STUDIES ON FASCIOLIASIS WITH SPECIAL REFERENCE TO FASCIOLA GIGANTICA IN EAST AFRICA

by

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I declare that this thesis has been composed by myself and that the work is my own except where indicated.

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SUMMARY

Following a general introduction, the extensive literature on fascioliasis is reviewed. The distribution, importance, pathogenesis and pathology of the disease are considered, with special emphasis on *Fasciola gigantica* infections.

Field studies are reported on the epidemiology and control of *F. gigantica* in East Africa and a survey described of infections with *Fasciola* spp. in African wild mammals. This survey was undertaken in order to assess the importance of wild mammals in the epidemiology of this disease, in view of the increasing development of game parks and ranching schemes.

The techniques used in the experimental studies are outlined with emphasis on those modified during this work. The techniques described include the cultural methods by which the infective agents were obtained, with special reference to the large scale production of metacercariae of *F. gigantica* and a detailed discussion of the problems which had to be overcome before this was possible. Other techniques described include those used in haematological examinations, faecal egg counts, liver function tests, serum enzyme assays and other biochemical studies.

The difficulties which had to be overcome to obtain and maintain experimental cattle and sheep free of intercurrent diseases in Kenya are discussed.

Observations are described which were made in the course of several experiments using a total of 35 sheep and 46 cattle in Edinburgh and in Kenya.

In Edinburgh the experiments comprised studies on the pathogenesis of acute and chronic fascioliasis (Fasciola hepatica) in sheep.

In Kenya chronic fascioliasis (F. gigantica) was studied in sheep with special emphasis on the economic importance of this condition. It was found that the infected animals gained weight more slowly than the uninfected controls and that this arose mainly from differences in the amount of body fat.

Several experiments were carried out in Kenya on cattle infected with single doses of 500, 1000 or 2000 metacercariae of F. gigantica or with two doses of 500 metacercariae ten weeks apart. The pathogenesis of the disease and the parasitological findings in these animals are described in detail. Other studies described include observations on long term chronic fascioliasis in cattle.

The gross pathology of F. gigantica infections in cattle from 2-51 weeks after infection is also described.

The main finding from these studies is that F. gigantica appeared to be less pathogenic for cattle than reported by earlier workers.

There was no evidence of an "acquired self-cure" under the conditions of these experiments, which would support the suggestion that resistance to F. gigantica

infections in cattle may be merely related to the fibrosity of the liver parenchyma. Further support was found for this suggestion from the study of the leucocyte levels and other observations.

Evidence was found that F. gigantica has a relatively short life in cattle.

The dissertation ends with a discussion of the results from the experimental infections.

SECTION I

Introduction and literature reviews

Chapter 1 Introduction

Chapter 2 History, distribution and importance of *Fasciola* spp.

Chapter 3 Pathogenesis and pathology of fascioliasis

CHAPTER 1

Introduction

Fascioliasis is the name given to the disease caused by liver flukes of the genus Fasciola. This dissertation will be mainly concerned with fascioliasis caused by Fasciola hepatica Linnaeus, 1758, and F. gigantica Cobbold, 1855. The former has been known since the Middle Ages and is one of the most widely recognized of all helminths, but the latter was only first described as a separate species in 1855. It largely replaces F. hepatica in most of the warmer countries of the world.

Both species require an intermediate snail host. F. hepatica is, throughout the greater part of its range, transmitted by Lymnaea truncatula or snails not readily distinguished from this on morphological or ecological grounds, and F. gigantica is transmitted by Hubendick's (1951) superspecies L. auricularia (Kendall, 1954) - the usual species in East Africa being L. natalensis.

This disease and its control is one of the major problems in veterinary helminthology, and its relative importance is increasing as many of the other diseases and conditions have been investigated and brought under control. However in the developing countries of Africa the absolute importance of F. gigantica infection is also likely to increase as livestock populations are increasing, with the control of the

great epidemic diseases such as rinderpest and contagious bovine pleuropneumonia and, under more developed conditions, east coast fever. This increase in numbers of hosts means that there is a greater chance for infection to build up at watering places, and also has increased the need for more watering places. Furthermore the increase in human populations and the increasing pace of development often necessitates the development of irrigation schemes for agricultural crops. Unless suitable control measures are taken these may become foci of infection because they are suitable habitats for the intermediate host of F. gigantica.

The increase in human populations also means that more land is needed for agricultural crops, and this is often acquired at the expense of traditional grazing areas. As the pressure on the available grazing increases, livestock are forced to use less suitable areas which may include areas infested with Lymnaea and contaminated with the metacercariae of F. gigantica. An example of this occurs when flood plains are grazed before the metacercariae have died off, such as described in Pakistan by Kendall (1953). Another result of this pressure on the available grazing is that livestock encroach more and more on to the land used by wild mammals, and these may then be important in the epidemiology of fascioliasis in these areas.

When this work started little was known about the

pathogenesis of F. gigantica infection in the domestic animals, and there was little published work relevant to Africa besides Sewell's (1962, 1966) studies on cattle in Nigeria. For this reason this aspect of fascioliasis was the main subject for study in Kenya, following the preliminary work on sheep infected with F. hepatica in Scotland. There were also very few references in the literature to this infection in wild mammals in Africa and a survey was undertaken in order to find those species which are naturally infected.

However, the epidemiology of fascioliasis had not been so neglected in East Africa, and both Coyle (1956, 1958, 1959, 1961) in Uganda, and Hammond (1965) in Tanzania studied this. Furthermore, detailed studies on the larval stages of the life-cycle of F. gigantica were published by Dinnik and Dinnik (1956, 1959, 1963, 1964), and the habitats and tolerance ranges of the snail host in Kenya described by Van Someren (1946). Both of these have important applications in the epidemiology of the disease in East Africa.

CHAPTER 2

History, Distribution and Importance of Fasciola Spp.

1. General History

As most of the early work was carried out with Fasciola hepatica, and indeed no other species of Fasciola was recognized until 1855, this review will deal mainly with this species.

The following species of Fasciola are considered valid; doubtful species will be referred to below.

Fasciola hepatica Linnaeus, 1758.

F. gigantica Cobbold, 1855.

F. nyanzae Leiper, 1910.

F. tragelaphi Pike and Condy, 1966.

The first two species were described by Dawes (1956) and Kendall (1965) and the latter two will be considered in Chapter 5.

F. hepatica was the first trematode to be described (de Brie, 1379) according to Faust and Russell (1964). Reinhard (1957) gave an historical account of the early investigations on this liver fluke, as well as references to some of the early epidemics, while Neumann (1892) gave more details of these and considered that the first epizootic mentioned in history is that which appeared in Holland in 1552. He presented evidence to indicate how important this disease was in Western Europe before any scientific control measures were applied. Thomas (1883) wrote that fascioliasis was

always common in certain districts of England and in many parts of the world, and that because of a succession of very wet seasons there was a serious outbreak in the winter of 1879-80 when an estimated 3,000,000 sheep died of this disease in the United Kingdom. As a result of this the Royal Agricultural Society of England offered Thomas a grant to undertake research into the life-history of the liverfluke, and he started work in June 1880.

The history of the work that had led up to the state of knowledge at that time has been described by Taylor (1937, 1964). Thomas (1883) described how he had succeeded in infecting L. truncatula and obtaining cercariae from them. His results were published in 1882, about three weeks after those of Leuckart (1882) who had worked independently in Germany. According to Faust and Russell (1964) this was the first complete life cycle of a digenetic trematode to be worked out.

The next requirement was to find the migratory route by which the young liver flukes arrive in the bile ducts, and this work has recently been reviewed by Dawes and Hughes (1964). Lutz (1893) infected laboratory animals and a goat and demonstrated the development of the adult flukes. He believed that the young forms were carried passively to the liver from the walls of the small intestine by the hepatic portal system. Although Lutz believed that he was working with F. hepatica, Alicata (1938) considered that he was

dealing with F. gigantica as this is the species of liver fluke prevalent in Hawaii where this work was carried out. However it is unlikely that the routes differ in the two species of flukes (Dawes and Hughes, 1964). Sinitsin (1914) first demonstrated that the young flukes pass through the wall of the intestines and across the peritoneal cavity to penetrate the liver. This migration of the juvenile flukes was later investigated by Schumacher (1938) who came to the same conclusions as Sinitsin.

Doubtful Species

The separation of the genus Fasciola into distinct species is often difficult because there are no associated and consistently distinctive anatomical features. As Kendall (1965) pointed out there is, in fact, great variation in the size of F. hepatica and F. gigantica depending on such factors as the age of the fluke, the species in which it is found, and the level of infection, very heavy infections tending to result in smaller flukes. In the survey of F. gigantica infections of wild mammals in East Africa, it was noticed that specimens from some species were much larger than from others, but there were not enough samples to indicate whether this was a constant factor. Fixation can have a profound effect not only on the size of the fluke, but also on the relative sizes of the various parts of the body, some of which are used in identification. A similar effect was seen during

the collection of Athesmia sp. (Dicrocoeliidae) from jackals (Canis mesomelas and C. adustus) in Tanzania as, when whole infected livers had been put into 10% formol-saline immediately after death, the Athesmia were very contracted and their measurements very different from those which had died before fixation (Hammond, to be published). Coyle (1961), in a series of photographs at five-second intervals, showed a single live F. gigantica both in its own typical form and also appearing very like a typical F. hepatica. It was also noticed in these studies that live F. gigantica which may look rather like F. hepatica, revert to more typical specimens when they relaxed after death.

Other features which aid the differentiation of the species are the size and shape of the eggs, and here again, it is likely that in such plastic organisms variations can occur, due to fixation and storage, and especially if the storage solutions are not isotonic. Eggs may alter in size and shape after prolonged storage in the gall bladder, and fully mature eggs may differ from those taken from the uterus. The egg size may also bear some relationship to the size of the fluke itself.

Therefore it would seem that too much reliance has been placed on minor differences in the anatomical features of flukes and eggs in the differentiation of new species. Other supporting factors should be

present before a new species is erected, such as a requirement of different snail hosts and differences in development in these as well as in the final hosts.

Flukes from cattle slaughtered at St. Louis, Senegal, were briefly described by Railliet (1895) as F. hepatica angusta, and specimens from cattle and buffalo in Egypt were described by Looss (1896) under the name of Distomum hepaticum var. aegyptiaca. However both Faust (1920) and Jackson (1921) regarded both these as being identical with F. gigantica, and Dawes (1956) lists them as synonyms under F. gigantica.

Sinitsin (1933) attempted to create two new species, F. californica and F. halli, which he differentiated from F. hepatica on the basis of very slight morphological differences in the adults as well as the number of generations of sporocysts and size of rediae. He thought that his two new species were separated from F. hepatica when the American continent was severed from Europe. In a review of American flukes, Price (1953) stated that while those from some areas appeared to be indistinguishable from the Old World F. hepatica, other lots approach F. gigantica in shape, and that in addition to these types there was in the Gulf Coast area a form that seemed to be intermediate between the F. gigantica and F. hepatica types. He suggested that as Gulf Coast ruminants harbour all three types there may be present a hybrid of F. hepatica and F. gigantica, as well as both these

species, but that it was possible that these three types may really be variations of a single species.

Another new species, F. indica, was proposed by Varma (1953) for the large species of Fasciola from India. This was challenged by Sarwa (1957) who considered this proposed species to be identical with F. gigantica. Furthermore, Kendall and Parfitt (1959) were unable to distinguish between flukes and eggs from Pakistan and from Africa.

In Japan, Watanabe (1962) claimed that infection with typical F. hepatica did not exist and that infections with typical F. gigantica were confined to a few districts and were not common. He thought that an intermediate species existed and that this was the same as F. indica. Generally speaking, the eggs and the adults of the Japanese liver fluke resembled F. gigantica, but it resembled F. hepatica in its developmental stages. Further, Oshima, Akahane and Shimazu (1968) after stating that the common liver fluke of Japan was always known as F. hepatica concluded, after studies on liver flukes and their eggs from one area of the country, that the Japanese common liver fluke is a single group within the range of variation presented by F. indica, F. gigantica and F. hepatica.

Kendall (1965) concluded from his studies that F. hepatica and F. gigantica are the only two valid species and that the morphological differences which

have been shown by different authors are attributable to normal biological variation, to the use of specimens from different host animals, or to differences in fixation and mounting. It has been found by Haiba and Selim (1960) that there are differences in size between Fasciola from different host species in Egypt.

However Kendall (1960, 1965) in reviews of this subject makes no reference at all to F. nyanzae which is also generally accepted as a valid species (Dinnik and Dinnik, 1961).

Hybridization

It has been suggested by Price (1953) that hybridization has taken place between F. hepatica and F. gigantica in the Gulf Coast area of the U.S.A., although he considered that this cannot be finally established until studies are made of progenies of single individuals of each type in cattle and sheep, as well as chromosome and serological studies. There are other parts of the world where hybridization may have occurred. For such hybridization to take place both F. hepatica and F. gigantica would have to be present in the same final host and this would only happen in those parts of the world where both flukes are found. De Jesus (1938) in the Philippines reported several such cases, and Alicata (1952) reported such a double infection in Hawaii. Some other countries where both the species of liver flukes

occur are Pakistan (Kendall, 1954), Kenya and the Republic of South Africa (Dinnik and Dinnik, 1959). In Turkey, Guralp and Simms (1960) reported that nearly every liver in which F. gigantica was found also contained F. hepatica. It is possible that hybridization of F. hepatica and F. gigantica has produced the intermediate part of the range of liver flukes seen in Japan.

2. Geographical distribution and importance

In many countries, the geographical distribution and importance of Fasciola has not been sufficiently studied to give more than very general indications. Limited surveys of a few animals or of those from a small area, or one where special conditions apply can be misleading, and such references should be interpreted with caution. Unless these limitations are realized a false impression may be given of the importance or otherwise of fascioliasis. Surveys are sometimes only carried out at a time when a dramatic increase in incidence has drawn attention to the disease, yet conversely the fact that no survey work has been carried out does not mean that the disease is of little importance. This is sometimes because suitably trained staff are not yet available to carry out these surveys in many of the developing countries where the main effort is still aimed at the eradication or control of the major epidemic diseases.

The incidence of fascioliasis is largely

controlled by the number of suitable final and snail hosts in an area, except where these are kept entirely apart. The incidence of the snail host can vary very markedly with the topography, which also influences the climate, and especially the rainfall, of the area. Furthermore river systems, such as the Nile, in otherwise desert areas may contain suitable localised snail habitats, and here too the extrapolation of limited surveys on fascioliasis to a national incidence must be avoided. Most countries seem to have areas where the incidence of fascioliasis is quite high, but it is often difficult or impossible to estimate how extensive these areas are and how important the disease is in each country as a whole. It must also be remembered when interpreting the figures given at meat inspection that the animals slaughtered may have come from another part of the country, or even from another country and this is especially common in Africa. Furthermore in some countries, such as Kenya and South Africa, where both F. hepatica and F. gigantica occur, no distinction is made between them at meat inspection and so such data cannot be used to determine their relative importance.

The importance of a disease like fascioliasis is very hard to assess. The usual information available is the percentage of all livers condemned for fascioliasis at the abattoir. The figure given often bears little relation to the actual number of animals

infected, as the livers are often not totally condemned in light infections but only the affected parts are removed (Condy, 1962). It is also easy to miss very light infections (Froyd, 1959), and the efficiency of detection will vary with the training and experience of the meat inspector. Other losses caused by chronic infection are a lower rate of weight gain, Condy (1961), poorer carcass quality and less wool of lower value. According to Ershov (1956) in relatively mild cases of fascioliasis, caused by either species in cattle, there is a fall of 10% in milk yield whereas more severe infections may cause a 20% fall. Further, Kendall (1954) reported that in certain areas of Pakistan work-oxen may have to be replaced every second or third year.

The disease is usually chronic in cattle but outbreaks of the acute disease have been reported. It would seem that the acute disease is more common in sheep and goats, and Condy (1962) thought that in Rhodesia fascioliasis is invariably acute in these animals. Neumann (1892) spoke of severe outbreaks which led to the abandonment of the sheep industry in part of Burma and Kendall (1954) found the same effects in some parts of Pakistan. Under these conditions cattle may replace the sheep as they are less susceptible to the acute disease.

In addition to these direct losses there are also the expenses involved in any necessary control

measures, such as cost of anthelmintics and the labour costs of mustering and treating livestock, fencing and draining dangerous areas and the cost of molluscicides and their repeated application. There is also the reduced efficiency in the utilization of grazing areas and watering places known to be dangerous. Lastly, there is the fact that fascioliasis is also a human disease although not often reported. It will be discussed later.

It is generally believed (Kendall, 1965) that the vectors of Fasciola are limited to species of Lymnaea, and Hubendick (1951) pointed out that while the Lymnaeidae as a whole cover most of the land surface of the world they are absent from the extreme north and south, and have not been reported from a wide area of northern South America, from parts of the Sahara and Arabian deserts, and from Western Australia and most of the Pacific Islands. However Hubendick (1951) pointed out that in the case of South America this may be due to lack of knowledge. This obviously limits the distribution of Fasciola.

(a) F. hepatica

According to Dawes (1956) F. hepatica is cosmopolitan in its distribution and Lapage (1962) also believed this to be so, as did Soulsby (1968). It is certainly widespread in its distribution, but is largely replaced by F. gigantica in most of the warmer countries of the world.

Little is known of the incidence of infection with F. hepatica in Africa as in most countries no distinction is made between it and F. gigantica at meat inspection. In Kenya Froyd (1959) in a survey of 1000 cattle only found F. gigantica, and concluded that F. hepatica, although occurring in Kenya, is uncommon, and Bitakaramire (1968) in a survey of livers condemned by the Kenya Meat Commission found the infection to be F. gigantica in all cases, although he pointed out that F. hepatica infection has been reported on occasions. In reporting on a survey in sheep and goats MacOwan (1962) stated that F. hepatica was largely restricted to the Molo-Mau Narok and the Kinangop-Ol-Kalau and Thomson's Falls areas. These are all cool high altitude areas. In Uganda, Coyle (1961) stated that this species had not been found and that it was unlikely that it occurred, but Bwangamoi (1968) thought that F. hepatica probably did occur. Hammond (1965) could find no evidence of F. hepatica in Tanzania.

(b) F. gigantica

According to Dunn (1969) this species occurs in many parts of Africa and Asia, including Asiatic Russia, in the southern U.S.A., and in Europe in Spain and European Russia. It would seem that Dunn considers ~~that~~ the flukes described by Price (1953) from the southern U.S.A. to be F. gigantica. Dawes (1956) stated that this species was found in America,

Asia, Africa and Europe but gave no supporting evidence. Soulsby (1968) considered that this was the common liver fluke of domestic stock in Africa and that it also occurred in the Indian sub-continent, Formosa, Hawaii and the Philippines.

The importance of F. gigantica in each country in East Africa will be considered separately.

Kenya

In a survey of slaughter cattle at the Kenya Meat Commission abattoir at Athi River, Froyd (1959) found that 35.4% of 1,000 cattle were infected with F. gigantica. In another survey (Froyd, 1960) 34.1% of 1,000 cattle were either infected with F. gigantica or had lesions which were assumed to be due to chronic fascioliasis although no parasites were found. Bitakaramire (1968) presented a table showing the annual number of bovine livers condemned because of liver fluke by the Kenya Meat Commission for 10 years between 1954 and 1966; 32.84% were condemned in 1966. No distinction was made in this table between those infected with F. gigantica and F. hepatica, but Froyd (1959) has pointed out that the latter is uncommon in Kenya. In 1966, the last year for which figures were available, Bitakaramire (1968) showed that the livers of 40.68% of 62,670 cattle owned by Europeans and 29.20% of 65,123 cattle owned by Africans, were condemned because of liver fluke infection. It is likely that the actual infection rate was higher

(Condy, 1962). All the flukes examined were identified as F. gigantica.

Tanzania

Fascioliasis in Tanzania is due to F. gigantica; F. hepatica has never been reported (Hammond, 1965). A survey of the incidence of fascioliasis was also conducted by Hammond (1965) in mainland Tanzania and he found that the proportion of cattle livers condemned for fascioliasis varied from 1% at Tabora abattoir to 46.8% at Iringa. Tarime and Musoma, with 38% and 37.2% condemnations respectively, were two other areas with high rates of condemnation. Again these figures are for condemnations only, and lightly infected livers which were trimmed but not condemned, are not included. Hammond (1965) also referred to severe losses due to fascioliasis, especially in the highland grass areas.

Uganda

The incidence of fascioliasis in Uganda has been investigated by Coyle (1956, 1961). Again, only infections with F. gigantica were recorded. He reported that the incidence of total condemnations of bovine livers from all causes in the five year period 1948 to 1952 varied from 14.7% to 46.9% at Kampala abattoir, and in the six year period 1951 to 1956 from 7.5% to 44.7% at Jinja abattoir. He commented that the cattle at both abattoirs included a variable number of animals imported from Kenya. Coyle (1961) stated that in the Teso district of Eastern Province the

incidence in livestock is almost 100%, as most of the grazing land is composed of grass swamps which contain good snail habitats. About 40% were found infected at Mbarara abattoir and about the same level of infection was found in cattle livers at Kabale abattoir. In the Mengo district the incidence in cattle was about 25% in some areas but very low in others. There is therefore no doubt that in some parts of Uganda fascioliasis is a very important disease of livestock.

CHAPTER 3

Pathogenesis and Pathology of Fascioliasis

Most of the work on this aspect of fascioliasis has been done with Fasciola hepatica but, except in a few instances, there is no reason to suppose that the work does not also apply to F. gigantica. Therefore the work done with F. hepatica will first be reviewed, and such studies as exist on F. gigantica will then be considered in more detail, and compared with the previously described findings for F. hepatica.

Extensive reviews on various aspects of the pathogenesis and pathology of fascioliasis have recently been provided by Sinclair (1967), Pantelouris (1965), Taylor (1964) and Dawes and Hughes (1964) from the very considerable literature on this subject which now exists.

1. Migration and development in the definitive host

Sinitsin (1914) first demonstrated that F. hepatica passes through the wall of the small intestine and enters the liver after crossing the peritoneal cavity, but most of the present knowledge of this phase of the life cycle is derived from the work of Schumacher (1938). The recent review by Dawes and Hughes (1964) covers these studies which showed that most of the young flukes reach the abdominal cavity about 24 hours after infection, begin to enter the liver after 48 hours and have entered the liver in 4 to 6 days. Whereas Schumacher used guinea pigs, Kendall

and Parfitt (1962) working with sheep reported that the flukes were in the peritoneal cavity approximately 18 hours after dosing, in the liver parenchyma 90 hours after infection, and that they took 40 days to reach the main bile ducts. The flukes contained eggs and therefore were considered to be mature by 43 days and the infection became patent with eggs in the faeces by 55 days post-infection.

Although the liver is the usual organ penetrated by the young fluke, there are many records of it becoming established in other locations, and occasionally it penetrates the visceral peritoneum or enters the blood stream and is carried to various parts of the body. Sinclair (1967) stated that the lung is probably the most frequently affected other organ. Prenatal infection in cattle has been reported by Enigk and Düwel (1959) but Taylor (1964) considered that it was a rare occurrence.

The means by which the flukes find the liver is unknown, but it has been suggested that a chemotaxis may be involved (Sinclair, 1967). According to Dawes and Hughes (1964) the fluke usually spends very little time wandering over the surface of the liver. Dawes (1961) presented a detailed study of the migration of the flukes in the mouse liver and believed that the food consumed at this time consisted of the liquid cytoplasm of hepatic cells together with leucocytes and some erythrocytes. He noted that the mouth of the

migrating fluke was almost invariably found pressing into the hepatic tissue and produced photomicrographs to show this.

According to Sinclair (1967), the time during which the flukes migrate in the liver parenchyma varies according to the species of host and is generally longer in larger animals. The manner in which the bile ducts are entered is unknown. Urquhart (1954) first noted flukes in the bile ducts of rabbits about 45 days after infection, and Dawes (1962) found that in the mouse entry into the bile ducts may take place after 24 days but more frequently only occurs after 29 to 32 days. In sheep Kendall and Parfitt (1962) found that the flukes reached the bile ducts after 40 days, and Ross, Todd and Dow (1966) found this period to be 49 days after infection in calves. Sogoyan (1956b) found that F. gigantica first reached the bile ducts in sheep 80 days after infection.

Sinclair (1962) first found eggs in the faeces of sheep during the tenth week after infection, but Boray (1967) found them at 56 days and Dixon (1964) after 61 days in sheep and 63 days in calves. With F. gigantica in Nigeria, Sewell (1962) first found eggs in the faeces of his experimental cattle 86 days after infection, considerably longer than the time given for F. hepatica. Furthermore Dinnik and Dinnik (1956) in Kenya found the shortest period to be 103 days with four calves which they had infected. Kendall and

Parfitt (1953) found the time required for the full development of a Pakistani strain of F. gigantica in a calf and in a sheep to be 110 days, and Alicata (1938) in Hawaii using a local strain found that eggs were first passed in the faeces of a calf 84 days after infection. In their work in Turkey, Guralp, Ozcan and Simms (1964) found that the shortest time for eggs to be passed in 17 infected sheep was 102 days, that one steer started to pass eggs after 89 days, one buffalo after 132 days and two Angora goats at 109 and 110 days respectively. Grigoryan (1958) reported that eggs were first seen in sheep faeces by 100 to 105 days after infection, and Ghani (1960) found the prepatent periods in two sheep to be 115 and 117 days.

It is apparent from these figures that F. gigantica takes appreciably longer to mature in the definitive host than does F. hepatica.

There is little evidence of the longevity of liver flukes in the final host. Certainly Durbin (1952) found that F. hepatica lived at least 11 years in sheep, and Leiper (1938) recorded at least 4 years 9 months in goats, while Montgomerie (1931) reported a period of at least 3 years and 1 month in rabbits. There are few references to the longevity of F. hepatica in cattle. Montgomerie (1931) noticed that, in slaughter houses, livers of 2½ year old cattle commonly show evidence of severe infection without the presence of a single fluke. Dixon (1964) reported

that the patent period in cattle was approximately 9 months, while Ross (1968) found that the life span of F. hepatica in cattle was a function of the level and pattern of infection, but that with low level infections it is at least 26 months although there is a significant loss of parasites by 21 months after infection. With F. gigantica, Alicata and Swanson (1941) showed that in cattle most of the flukes were eliminated by the end of one year, but that some of them may survive for at least 3 years and 4 months. This fluke has been found to live for at least 5 years and 56 days in a giraffe (Giraffa camelopardalis).

2. Chronic fascioliasis

According to Soulsby (1968) the main lesion is a progressive biliary cirrhosis which ultimately produces a hard fibrotic liver, with bile ducts which are prominent, thickened, fibrous, and, in some host species, calcified. The fibrosis is produced by the repair to the migratory tracts and a cholangitis. The walls of the main bile ducts are markedly thickened and the ducts are dilated. The epithelium is extensively damaged by the flukes and may become completely denuded despite considerable hyperplasia.

The histopathology of the infected liver has been studied in a variety of experimental animals as follows: in the mouse by Dawes (1961), and Sewell (1962); in the rabbit by Urquhart (1956); in the rat by Thorpe (1965); in the sheep by Dow, Ross and Todd (1968);

and in the calf by Dow, Ross and Todd (1967). Studies on the pathology in cattle were also carried out by Morill and Shaw (1942). Stemmerman (1953) described the pathology of human and guinea pig livers infected with F. gigantica.

There are broad similarities in the liver pathology of fascioliasis in all these species, but considerable variation in severity occurs (Sinclair, 1967), while in most host-species there is little inflammatory reaction to the migration of the flukes. Dawes (1963) observed cytoplasmic outgrowths on the free surface of the epithelial cells of the bile ducts long before the flukes arrive, and by the time the flukes entered the bile ducts the epithelium is extensively folded. However Sinclair (1967) pointed out that proliferation of the bile duct epithelium, independent of changes in the parenchyma, is a well recognized non-specific lesion in liver pathology. Taylor (1964) pointed out that in mild cases the bile duct enlargement is often localized, but, in more advanced cases, the thickening of the bile ducts extends to all the major branches, while Jubb and Kennedy (1963) considered that the dilation of the ducts was due largely to the mechanical distension by masses of flukes and bile. Sinclair (1967) believed that most of the immature flukes in the liver reach the bile ducts, but some may become enclosed in fibrous cysts in the parenchyma. These fibrous 'nodes' are frequently seen in cattle livers

infected with F. gigantica in East Africa.

Coyle (1961) considered the pathological significance of F. gigantica in natural infections in cattle in Uganda, and Sewell (1966) thought that, in general, his description suggested that East African cattle react more severely to the infection than Nigerian cattle. The lesions of F. gigantica were described by Guralp, Ozcan and Simms (1964). About 90 days after infection in sheep the flukes were moving into the bile ducts and the haematomas, which were a marked feature of the infection, began to resolve. Deposits of salts lined the bile ducts containing flukes in some cattle, but were not found in similar bile ducts either in sheep or buffalo. Davtyan (1956) found F. gigantica to be less infective but far more pathogenic to sheep and rabbits than F. hepatica, whereas it was more infective but less pathogenic than F. hepatica in cattle. Sinclair (1967) stated that the pathogenesis of F. gigantica had been inadequately investigated, but was generally considered to be similar to F. hepatica.

In sheep F. gigantica has a slower rate of development and greater pathogenicity than F. hepatica (Sogoyan, 1955, 1956a and 1956b) and the peak of traumatic damage with the latter occurs at 45 to 60 days after infection, and by 68 to 70 days most of these flukes have entered the bile ducts, whereas the most traumatic period with F. gigantica infection is

just beginning. Some flukes reach the bile ducts by 80 days after infection, but Sogoyan often found that this was delayed up to 120 or 130 days. Condy (1962) also believed that F. gigantica is more pathogenic in sheep, and pointed out that when these flukes have reached the size of adult F. hepatica they are still migrating in the liver parenchyma. Sewell (1966) considered that F. gigantica is better adapted as a parasite of cattle than is F. hepatica. Macroscopically this can be seen, since the heavy fibrosis and calcification caused in the livers of cattle infected with F. hepatica is less marked in those infected with F. gigantica, so that the livers appear similar to those of sheep infected with F. hepatica.

Sinclair (1967) referred to the work of several authors who have studied the chemical composition of the blood of animals with fascioliasis. He stated that the changes in serum proteins are similar in all host species, the initial hyperglobulinaemia being accompanied later by hypoalbuminaemia. Weinbren and Coyle (1960) reported a marked fall in the albumin-globulin ratio of the serum protein in cattle naturally infected with F. gigantica. Sewell (1966) found bilirubinaemia and jaundice to be features of the later stages of sub-acute fascioliasis due to F. gigantica in a steer, but Ross, Todd and Dow (1966) failed to demonstrate any deviation from normal in serum bilirubin levels in two calves, however these were much

less heavily infected with F. hepatica and did not develop sub-acute fascioliasis. On the other hand Roberts (1968) demonstrated a rise in serum bilirubin content in sheep with acute fascioliasis due to F. hepatica.

Determinations of various enzymes in the blood, and also dye excretion tests have been used by several workers to assess the degree of tissue damage and impairment of liver function. Serum glutamic oxaloacetic transaminase (S.G.O.T.) levels have been used by Ross, Todd and Dow (1966), Sinclair (1966), Thorpe (1965), Thorpe and Ford (1969), Roberts (1968), Pullan (1968) and Sewell (1967) for such assessment. Thorpe and Ford (1969) also found increases in the levels of sorbitol dehydrogenase (S.D.) and glutamic dehydrogenase (G.D.) in infected sheep. Sewell (1967) and Pullan (1968) also found increases in the G.D. levels in infected sheep. The bromsulphthalein (B.S.P.) dye excretion test has been used by Sewell (1962), Sinclair (1966), Pullan (1968) and Roberts (1968) and increased clearance times were demonstrated during the acute stage of the disease. According to Sewell (1966) if the host survives until the flukes have passed from the liver parenchyma into the bile ducts, the parenchymal liver damage is rapidly repaired. Thereafter any dysfunction, which may have been detectable by liver function tests during the sub-acute phase, soon returns to normal.

Several workers have believed that toxic substances are responsible for some of the pathological changes in fascioliasis; Sogoyan (1955) working with F. gigantica was one of these. Ershov (1956) referred to the release of toxins by the parasite which were responsible for both local and general changes, and he also considered that the parasite's metabolites were toxic. Poljakova-Krusteva, Krustev, Ellis and Bird (1968) considered that a toxic secretion from the fluke plays a major part in the morphogenesis of the liver lesions in fascioliasis. Other references to toxins or toxic metabolites of Fasciola are those of Stemmerman (1953), Jubb and Kennedy (1963), and Flury and Leeb (1926) who concluded that the parasite's secretions were toxic. There is, however, little precise information available concerning such toxic production by the fluke.

Sewell (1962, 1966) reported detailed observations on F. gigantica infections in cattle in Nigeria, and Bitakaramire (1968, 1969) and Bitakaramire and Bwangamoi (1969) reported accounts of their work with F. gigantica in cattle in Kenya.

Davtyan (1956) referred to deaths in sheep with as few as 33 F. gigantica, and Guralp, Ozcan and Simms (1964) reported the macroscopic lesions seen at autopsy in sheep.

A reduced live-weight gain in lambs infected with low numbers of F. gigantica was reported by Condy

(1961), and Sewell (1966) found that in cattle infected with F. gigantica each fluke reduced the annual live-weight gain by about seven ounces.

3. Acute fascioliasis

Acute fascioliasis may be defined as fascioliasis which results in the death of the host during the migratory stage of the parasite. Sinclair (1967) considered that the primary lesion is a traumatic hepatitis with haemorrhage caused by simultaneous parenchymal migration of a large number of parasites. The effect on the host is governed by the numbers of invading flukes, the time over which the infection is acquired, the susceptibility of the host and the size of the liver. With high level infections of F. hepatica in calves the majority of the flukes are trapped and eliminated in the parenchyma (Ross, 1965, 1967). Ross (1965) found that infections of between 2,500 and 15,000 metacercariae of F. hepatica in calves resulted in few adults in the bile ducts, the majority of flukes becoming inhibited and subsequently encapsulated in the liver parenchyma. He also suggested that this inhibition may explain the rare occurrence of the acute disease in cattle. However this does not appear to be so marked in cattle infected with F. gigantica as after infecting a bullock with 20,000 metacercariae Sewell (1966) recovered about 35% as young flukes. Also Bitakaramire (1968) found a mean recovery rate of about 24.7% from five calves each

of which had been given 10,000 metacercariae of F. gigantica and did not refer to any inhibition.

Both Sewell's and Bitakaramire's calves died during the migratory stage of the disease.

In his review of the literature on acute fascioliasis, Pullan (1968) stated that this syndrome can be relatively easily produced in laboratory animals by low doses of metacercariae. For sheep, however, infections with between 3,000 and 10,000 metacercariae were considered necessary by various authors (Jubb and Kennedy (1963), Taylor (1964), Ross, Dow and Todd (1967)). It would thus seem that F. gigantica is very much more pathogenic for sheep than F. hepatica, for Sogoyan (1956a) reported that all five sheep he infected with 600 metacercariae died of acute fascioliasis, the numbers of flukes recovered ranging from 56 to 190. Sogoyan (1955) found that six sheep aged 1½ to 2 years, given 10 metacercariae per kilogramme body weight, also died from acute fascioliasis 70 to 90 days after infection. However, Grigoryan (1958) reported that an infective dose of 160 to 270 metacercariae produced only the chronic infection in his sheep. F. gigantica also appears to be very pathogenic for goats and Hammond (1965) stated that as few as 42 flukes were fatal by 94 days after infection. Condy (1962) reported from Rhodesia that fascioliasis is invariably acute in field outbreaks in sheep, and Mackinnon (1964) stated that the vast majority of these deaths occur

when the flukes are 2 to 3 months old. Guralp, Ozcan and Simms (1964) gave some details of their findings at autopsy in sheep experimentally infected with F. gigantica. They also reported deaths between 73 and 113 days after infection with as few as 100 metacercariae.

Histopathological changes in the liver of sheep with acute F. gigantica infection were described by Sogoyan (1955, 1956b), while Ross, Dow and Todd (1967) detailed those of the acute disease caused by F. hepatica.

Boray, Happich and Andrews (1965), Kendall and Parfitt (1962) and Ross, Dow and Todd (1967) stated that deaths from acute fascioliasis due to F. hepatica in sheep did not occur until 7 to 8 weeks after infection. As described above F. gigantica takes 2 to 3 weeks longer than this, although the numbers of flukes were very much smaller. However, Sogoyan (1955), who infected 7 to 8 month old lambs with 5,000 metacercariae of F. gigantica each, killed them up to 56 days later; and Guralp, Ozcan and Simms (1964) implied that two sheep, given 1,825 and 2,073 metacercariae respectively, lived for at least 72 days after infection.

Pullan (1968) gives detailed references to the clinical picture and pathogenesis of acute F. hepatica infection, as well as the post-mortem findings. Infections with large numbers of metacercariae of

F. hepatica have been shown by several authors (Ross (1965); Taylor (1964); Ross, Todd and Dow (1966); Ross, Dow and Todd (1967); Kendall (1967); Kearney, Connolly and Downey (1967) and Roberts (1968)) to result in overcrowding or competitive inhibition and consequent stunting of the flukes. Taylor (1964) discusses the association between Black Disease, caused by Clostridium oedematiens and fascioliasis.

4. The anaemia of fascioliasis

Fasciola hepatica has been commonly referred to as a "blood sucker", the inference being that the anaemia associated with the disease results from the feeding habits of the parasite, and is in effect haemorrhagic in origin. Various authors have suggested other aetiologies including toxic agents (Ershov, 1956 and Flury and Leeb, 1926) or dyshaemopoiea (Sewell, 1966 and Sinclair, 1964). However Moroshkin, Kostina, Ivanski and Sutyagin (1964) indicated that erythropoiesis was not interfered with, while Symons and Boray (1967) concluded that the rate of erythropoiesis was greatly increased.

Furthermore, Sewell (1966) considered that there was no evidence that flukes produce a lytic toxin, as haemosiderosis or haemoglobinaemia are not features of fascioliasis, and jaundice is strictly a terminal symptom in the chronic disease. Furthermore he also found that the mean red cell fragility remained normal in all the animals infected with F. gigantica, although

the range of fragility was increased in the anaemic animals; this was probably associated with the anisocytosis and poikilocytosis observed. Sewell (1966) also considered that if a dyshaemopoietic anaemia was involved this could be because of a dyshaemopoietic toxin produced by the parasite, because the fluke was in some way depriving the host of an essential haemopoietic factor. He pointed out that Obara, Sonoda and Watanabe (1964) had recently shown that F. gigantica could preferentially absorb Vitamin B12 and thought this might be one cause of the anaemia. However, Sinclair (1967) has since shown that the parenteral administration of this vitamin or iron had no effect on the anaemia of sheep infected with F. hepatica.

Dawes and Hughes (1964) thought it odd that none of the investigators who had postulated or supported the hypothesis that adult Fasciola are "blood suckers" had ever shown how they can obtain the blood. Also Sinclair (1969) stated that nobody had been able to show definitely that flukes suck blood. There is, however, evidence to show that erythrocytes, haemoglobin and related pigments are to be found in the caecae of adult flukes (Flury and Leeb, 1926; Hsü, 1939) and, from his histochemical observations on the flukes' caecal contents, Stephenson (1947) concluded that they feed mainly, if not exclusively, on blood. Todd and Ross (1966) reached the same conclusion after their

studies on the caecal contents of adult flukes, and so also did Grembergen (1950) who found degraded haemoglobin in the caecae. Other opinions regarding the nutrition of the adult fluke include those of Lagrange and Gutmann (1961), who considered that they feed on the contents of the bile ducts, and Dawes (1963a) who described the flukes as feeding on the hyperplastic epithelium of the bile ducts.

In recent years there has been considerable evidence derived from experiments using radio-active tracers, to the effect that large quantities of red cells, or the breakdown products of such cells, are demonstrable in the faeces and bile of animals with chronic fascioliasis (Jennings, Mulligan and Urquhart, 1956; Pearson, 1963; Symons and Boray, 1967, 1968; Sewell, Hammond and Dinning, 1968; Holmes, Dargie, MacLean and Mulligan, 1968). However, Todd and Ross (1966) reported that in cattle the relative activities of fluke and bile in animals receiving red cells labelled with ^{51}Cr may indicate that they ingest blood but cannot be regarded as conclusive evidence of haematophagia by the parasite being the sole cause of the anaemia. These authors found that the initial loss of radio-activity and the excretion of label in the bile are severe handicaps in the use of this technique to study any blood-sucking activities of the parasite. However, similar studies using labelled Di-isopropyl phosphorofluoridate ^{-32}P, which does not

elute from sheep red cells, also showed that massive random loss or destruction of red cells occurs in chronic fascioliasis in these animals (Sewell, 1967; Sewell, Hammond and Dinning, 1968). At the same time there is evidence of loss of plasma protein which is relatively greater than the loss of red cells (Dargie, Holmes, MacLean and Mulligan, 1968) and these same authors described electron-microscopic studies showing that breakdown of the junction between the epithelial cells lining the infected bile ducts occurs, thus permitting the leakage of protein molecules.

Sinclair (1962, 1964, 1965), after comparative studies on infected and bled sheep, concluded that haemorrhage is not the main factor in the aetiology of the anaemia of fascioliasis. He further suggested that the anaemia is secondary to a disorder of the reticulo-endothelial system which leads to decreased erythrocyte production and increased erythrocyte destruction. Sinclair (1967) further thought that this reticulo-endothelial dysfunction may be caused by the liberation by the parasite of some unknown toxic substance.

Symons and Boray (1967) concluded that the extensive haemorrhages in the substance of the liver and blood-stained peritoneal fluid indicated that anaemia in the acute stages of the disease in sheep was due to blood loss. Sewell (1966) considered that it is possible that the later onset of the anaemia of

chronic fascioliasis, as compared to that of sub-acute fascioliasis, is merely related to the smaller numbers of flukes involved and to the greater size of the flukes in the bile ducts, as compared with the earlier forms in the parenchyma. Ross, Dow and Todd (1967) found the general features of acute haemorrhagic anaemia at autopsy of their sheep with acute fascioliasis. However, Pullan (1968) from radio-tracer studies suggested that there is a relative dyshaemopoiea and possibly a terminal haemolytic effect in acute fascioliasis in sheep.

5. Immunity

Dawes and Hughes (1964) concluded that there was little evidence from the literature of any significant naturally acquired resistance to F. hepatica. In his review, Sinclair (1967) also discussed this question and his findings agreed with those of Dawes and Hughes and he also stated that there was no evidence of any protective immunity against F. gigantica. Hughes (1963) could find no evidence of resistance in mice, rabbits or sheep following the administration of irradiated metacercariae of F. hepatica. However Bitakaramire (1968) in his discussion on an abattoir survey in Kenya considered that age immunity, or more likely, an acquired resistance to reinfection with F. gigantica had occurred in many cases. Coyle (1961) in discussing the occurrence of cattle livers with lesions of chronic fascioliasis, but containing no, or

very few F. gigantica, deduced that some barrier had been presented to reinfection. However, Sewell (1966) thought it more likely that the purely physical barrier afforded by the gross fibrosis was responsible for the absence of re-infection.

The various immunological techniques that have been used in studies on fascioliasis, and the results of these studies were adequately described by Dawes and Hughes (1964), Pantelouris (1965) and Sinclair (1967). Dawes and Hughes (1964) concluded by stating that it appeared that the prospect of immunizing the hosts of F. hepatica against liver fluke disease was poor at the present state of knowledge and techniques.

Sewell (1962), Pantelouris (1965) and Sinclair (1967) have reviewed the serological tests used for the diagnosis of fascioliasis and Sewell (1964) has shown that F. hepatica and F. gigantica can be distinguished serologically. Sinclair (1967) believed that there was little doubt that the antibodies detectable by serological and skin tests are without protective properties.

6. Host susceptibility

(a) F. hepatica

According to Soulsby (1968) this fluke occurs in the sheep, goat, ox and other ruminants, pig, hare, rabbit, beaver, coypu (Myocaster coypus), elephant, horse, dog, cat, kangaroo and man. It also infects the mouse (Dawes, 1961; Sewell, 1962; Boray, 1963),

the rat (Thorpe, 1965; Boray, 1963) and the guinea pig (Boray, 1963). In Britain the rabbit is an important reservoir of the infection (Taylor, 1964). Infections in wild mammals in Africa will be referred to in Chapter 5. A recent new laboratory animal is the gerbil (Helfer and Knapp, 1968).

Human fascioliasis, mostly due to F. hepatica, is reviewed by Dawes and Hughes (1964), and Facey and Marsden (1960) described an outbreak in England and compiled a comprehensive list of references. Taylor (1961) believed that many cases in man were either free from symptoms or had very slight symptoms and underwent spontaneous cure. Hardman, Jones, Davies and Watkins (1969) reported an outbreak where 37 people had become infected, the largest recorded in Britain, while Bendezu (1969) found that 60% of schoolchildren had F. hepatica eggs in their faeces in one mountain valley in Peru. Louw and Wilkie (1956) reported two human cases of F. hepatica infection in South Africa, while a large outbreak involving over 500 cases was reported in France by Coudert and Triozon (1957) when, as in many cases of human infection, contaminated water cress was implicated.

The well recognized variation in susceptibility to infection shown by different host species has been investigated by Ross, Dow and Todd (1967) who found that pigs had considerable natural resistance to infection while cattle were less resistant than pigs

but nevertheless more resistant than sheep or laboratory animals. Ross (1967) believed that the essential basis for these differences in susceptibility was due to differences in the fibrous structure of the liver.

(b) F. gigantica

According to Sinclair (1967) this parasite occurs in sheep, goats, cattle, horses and many wild mammals. Infections in wild mammals in Africa will be referred to in Chapter 5. Brumpt (1936) listed buffalo, cattle, goat, man and sheep, and Alicata (1938) gave a reference to the infection in a horse in Hawaii. Other references to the infection in large animals are those of Karib (1962) who found that camels are very rarely infected and then with only one or two parasites. Alicata and Bonnet (1956) reported the infection in wild pigs in Hawaii, and Alicata (1938) infected a pig experimentally with 300 metacercariae and recovered 84 flukes which were 20 to 28 millimeters long when he killed it 64 days later. Thus the pig would seem to be a better host for this parasite than for F. hepatica (Ross, Dow and Todd, 1967). Varma (1953) also obtained F. indica (F. gigantica) from a pig (Sus cristatus) in Singapore. Kendall (1965) considered that the water buffalo is perhaps the best host for F. gigantica, and Boray (1963) thought that cattle were the best hosts for anthelmintic tests against F. gigantica. Davtyan (1956), who found this parasite

to be more infective but less pathogenic to cattle than sheep, thought that it should be considered specific to the former host.

Human infections are apparently rare and Hammond (1965) reported that it had not been recorded in Tanzania; also there have been no reports from Uganda (Coyle, 1961). Faust and Russell (1964) only recorded human cases from Senegambia, Tashkent (U.S.S.R.), Vietnam and Hawaii. In Tashkent, Pigulewsky (1928) reported two cases, Segal, Humphrey, Edwards and Kirby (1968) recorded infections from Vietnam, while the report from Senegal was by de Govea (1895). Other records are from Iraq by Fattah, Babero, Karaghouli and Shaheen (1964), and from Cameroon by Rousset, Paraf, Trad and Benchetrit (1968). The latter authors claimed that F. gigantica had not been described before in man "en Afrique Noire". Goldsmid (1968) also reported a case in Rhodesia. However, most reported cases have been from Hawaii where Stemmerman (1953) described three cases, including details of the histopathology, while Alicata (1953) gave a summary of 19 cases. Alicata and Bonnet (1956) considered that infested water-cress was the most likely source of human infections. Alicata (1953) found that F. gigantica could reach maturity in man and lay eggs, but Stemmerman (1953) observed that most of the flukes are infertile and they have been recovered from such aberrant sites as the subcutaneous

tissues, the upper respiratory system, peritoneal cavity and once, "plucked from the external ear"! Stemmerman (1953) further considered that abortive infections may occur in man. This may be why there have been so few reports of human infections, for the chances of infection are probably high in some parts of the world.

SECTION II

Epidemiological studies

Chapter 4 Observations on the epidemiology of *Fasciola gigantica* infection in East Africa

Chapter 5 Infections with *Fasciola* spp. in African wild mammals

CHAPTER 4

Observations on the Epidemiology of Fasciola gigantica Infections in East Africa

In Tanzania, Hammond (1965) studied the epidemiology of Fasciola gigantica infections. One such outbreak of disease was caused by the dispersal of infected snails on to grazing land when the Mori river flooded in North Mara district. Many hundreds of animals were reported to have died. In another outbreak at Kisesa near Mwanza a small dam had overflowed during the rains and flooded some adjacent rice fields. Livestock normally watered at this dam and it was also a Lymnaea natalensis habitat. After the rice had been harvested, the area was opened for grazing and livestock picked up a very heavy infection. When these dry rice fields were examined during the outbreak many hundreds of L. natalensis shells were found. One stockowner reported that 79 cattle out of an original herd of 119 had died, as well as 55 out of 60 sheep and 3 out of 5 goats. Others in the same village reported similar losses.

In West Shinyanga springs feed small pools which are generally about 15 or 20 metres in diameter, and the overflow is drained away by a small stream which dries up after a short distance. These pools, and especially the first few metres of the outlet streams, were often heavily populated with L. natalensis. From a 2 metres length of one outlet stream, 86 specimens of

L. natalensis were collected and 28% of these were infected with Fasciola. Cercariae are probably carried downstream and encyst on the grass in the wet area where the stream ends. This green grass is very attractive for livestock especially in the dry season.

In Kenya an investigation was carried out into an outbreak of fascioliasis on a farm at Maseno near Lake Victoria. This investigation had to be carried out in April, during the wettest time of the year which was unfortunate because some of the habitats of L. natalensis were found to be flooded at that time.

Evidence of fascioliasis in the cattle

All cattle on the farm were Jersey-type animals. A cow died in November and at autopsy a veterinary surgeon had found the liver heavily infected with F. gigantica. It was not, however, considered that this infection was the cause of death. In February an 8 year old cow which was culled because of infertility was found to be infected with liver flukes, and in March a 15 year old cow was also found infected when culled for the same reason. In this month 11 of the 12 cows in this herd had Fasciola eggs in their faeces; the range was 3-45 eggs per gram (e.p.g.), with a mean of 19 e.p.g. All the cattle were reported to have been treated monthly with Minel (I.C.I.) but there was some doubt on both the dose rates and the timing used, and the evidence of infection showed that the control measure had been ineffective. In April faeces samples

were collected from 13 calves born between August and October of the previous year, and only one was found to be passing Fasciola eggs (5 e.p.g.). These calves were also reported to have been treated in the same way as the cows, but again there was some doubt as to the regularity of the treatment.

Physical features of the farm

The farm is completely enclosed with a post and wire fence. It consists of a series of ridges running south towards Lake Victoria. There is a permanent small swiftly-flowing stream in one of the valleys between the ridges and this is referred to as site 3. There is an earth dam on the west side of the farm buildings, away from the main grazing area and this is site 4. The grazing along the ridges and valleys was unimproved and contained a lot of low bushes and rough coarse grasses. There are also subsidiary streams and small seepage areas.

On the east side of the farm is the main grazing area where the cows spent most of their time. A panoramic view of this is shown (Plate 4.1). The main road is about 100 metres beyond the fence at the far left side of this view. To the right of this fence is about 70 metres of well drained grazing which slopes from the left to the right, and which adjoins a patch of badly drained coarse grasses. This is followed by a spring which immediately enters a cylindrical concrete well which has been built up to protect it. (Plate 4.2).

Plate 4.1. Panoramic view of the grazing at Maseno farm.

Plate 4.3. The concrete drinking trough (site 2). A Lymnaea natalensis habitat.

Plate 4.2. The cylindrical concrete well (site 1). Lymnaea natalensis were plentiful in this habitat.

This small well or trough (site 1) overflows into a narrow brick-lined water course which crosses to the right of the view and leads to the concrete drinking trough (Plate 4.3) which is site 2. The water course was in a bad state of repair and was not large enough for the flow of water, so that there was considerable overflow all along it with an accompanying growth of various water grasses. The drinking trough however was in good repair, and the overflow drained away through a fence into cultivated land which was inaccessible to the cattle.

Results of the survey

Only snails over 6 mm. in length were collected and examined for infection as it is unlikely that the infection would have been developed enough to be easily seen in smaller ones. All snails were preserved in 10% formol-saline and examined several days later. As fixation was not very satisfactory it is likely that infections at the sporocyst stage were missed, certainly none were found. It is therefore likely that infection rates were higher than are recorded here. The only parasites found in these snails were Fasciola sp. and in none of them was there any evidence of any other parasites, either internal or external.

Site 1 (the small well or trough). Here there were numerous L. natalensis of all sizes, together with egg masses. Forty-eight snails were collected at random over a period of a few minutes and 9 were

infected (18.7%). Three of these snails also contained mature cercariae.

Site 2 (the drinking trough) (Plate 4.4). Here the snails were also numerous and of all sizes, together with egg masses. Of the 164 snails collected as previously, 28 were infected (17.0%). Nine of these also contained mature cercariae.

Site 3 (the swiftly-flowing stream). Snails were not plentiful along this stream. Of the 31 collected over about one hour only one was infected (3.2%). Plate 4.5 shows small pools of still water and seepages, out of the main current, where L. natalensis were found.

Site 4 (the dam). Here snails were hard to find. Only 8 were collected over a period of about one hour and none of these were infected. Plate 4.6 shows the dam, and also the drinking trough below it where cattle water. The latter is cited as good sanitary practice to prevent infection. However cattle from outside the farm entered the dam to drink and thus contact was established between cattle and snails. This dam is fed by a stream which passes through closely planted eucalyptus trees, some of which had recently been cut down (Plate 4.7) at the entry point into the dam. The reason for the small number of snails which were able to be found in the dam was possibly that the water was tainted by the eucalyptus trees. Coyle (1961) found no snails of any species in streams flowing through

Plate 4.4. Another view of site 2. Lymnaea natalensis can be seen along the water line in the drinking trough.

Plate 4.5 The stream (site 3). A few Lymnaea natalensis were found in this habitat.

Plate 4.6. The dam (site 4) and trough below it where the cattle water. Very few Lymnaea natalensis were found in this habitat.

Plate 4.7. Eucalyptus trees at the entry point of the stream into the dam (site 4).

eucalyptus trees.

The sequence of infection on the farm

Sites 1 and 2 are considered together as they are part of the same ecological picture. Faeces of infected cattle dropped above site 1 (to the left of it in Plate 4.1), where the soil is badly drained, would not dry out - at least during the rainy seasons. Fluke eggs would hatch especially during heavy rainstorms which are common here. These rainstorms lower the temperature rapidly and Coyle (1961) considered that this is the main stimulation to hatching in the field. The miracidia would be washed by the rain into the well and then the current would carry them right along the system into site 2. Snails can therefore become infected at any point from site 1 to site 2. Infection of cattle probably occurs mostly when they come to drink in the trough, although they were also able to drink at site 1. There is no other place where cattle can drink in the area. It is possible for cercariae to encyst on grass round site 1, and between it and site 2 and these are also likely sources of infection. Cercariae can also encyst on the surface of the water (Dinnik and Dinnik, 1959) although it is not known for how long the metacercariae will float, Standen (1963) merely saying that they sink to the bottom when disturbed. However Boray (1963) found that about 10% of cercariae of Fasciola hepatica encyst on the watersurface by enclosing small

airbubbles which enable them to float for long periods and thus infect hosts through drinking. In the drinking trough it is unlikely that the water will be so disturbed that metacercariae, which have sunk, will be resuspended and therefore available for infection, as cattle are not able to enter it. In a further experiment to be described it was found that metacercariae less than 3 hours old were infective to sheep, although the recovery rate of adult flukes was lower than with those 2 to 7 days old. It is therefore possible for infection to take place in this way when the cattle drink first thing in the morning especially with metacercariae shed the previous night. LeRoux (1957) considered that when no vegetation is present in the snail habitat, such as a cattle watering trough, the cercariae encyst on the surface of the water and are swallowed when the animal drinks.

Site 3 is part of a separate water system. The rocky part of the stream cannot be entered by cattle as the sides are steep and it is very narrow. However they can enter further upstream where the banks are flat and sandy. Infected faeces and miracidia are washed down by the current to the rocky inaccessible part of the stream to infect L. natalensis, which live among the rocks in small pools which are backwaters of the main stream. It is also possible for snails to get infected upstream in the sandy area and be carried by the current, or to migrate, downstream. However,

as the cattle cannot reach the infected snails or the vegetation in their vicinity, and the current carries any floating metacercariae out of the farm, this habitat was not thought to be of much importance in the epidemiology of fascioliasis. L. natalensis was also found well above the water level of the main stream, in tiny seepages which trickled from the rocks. These snails would not be killed and would quickly re-infest the stream if molluscicide was only poured into the main body of the stream, so it should also be sprayed along the banks in any attempt to use this method of control in this type of habitat.

The small seepage areas, which were referred to in the description of the physical features of the farm, dry out at certain times of the year so that they are not thought to be sites where transmission can occur. They were very wet at this time, because of the heavy rains, which reduced the efficiency of the search for snails in these areas. No L. natalensis were found and there were places where the red-brown scum, due to 'iron bacteria' (Van Someren, 1946) was seen and such areas are unsuited to L. natalensis (Hammond, 1965).

The dam (site 4) is separate from any other water system on the farm. It has already been described. Although it was not found to be a transmission site it could become one if local cattle continue to water in it, and if the population of L. natalensis increases as could happen if the eucalyptus trees were cut down

and removed.

Control measures

The control and elimination of fascioliasis on this farm is quite simple and not expensive. The most important transmission sites (1 and 2) could be eliminated by treating the small well at site 1 and the surrounding area with a molluscicide such as copper sulphate which would then diffuse through the water system to the drinking trough at site 2. This trough should also be drained and scrubbed out to remove metacercariae and dead snails. Periodic examination and possible retreatment would also be necessary. No action is required for site 3 (the swiftly-flowing stream), and it is only necessary to prevent local cattle from entering the dam (site 4) so that it will not become a focus of transmission. This would be easy as there are alternative watering places, and the dam is near the farm buildings so that enforcement should not be difficult.

All cattle on the farm should be treated under proper supervision with a fasciolicide, three times at monthly intervals, and faecal samples should also be examined at the end of that time and monthly thereafter until it is clear that all transmission has stopped.

A further survey would have been desirable 6 months later, during the dry season, to see if these measures had been carried out and if they had proved

successful, and also to re-examine all the *L. natalensis* habitats at that time. However this was not possible.

Another study was made on the possible dangers of *F. gigantica* infection in an area where it was proposed to establish a cattle ranch. Here the climate and conditions are very different from the previous investigation. The topography is of undulating grassland, west of Nairobi near Athi River village, the river itself forming one boundary of the property. The climate is much drier than at Maseno. There are wooded drainage lines between the hills but only the one permanent river.

The area was visited on two occasions and a snail survey was carried out on all the water bodies on the proposed ranch. Because of the partial failure of the long rains in April-May there was very little standing water when the survey was carried out in June and July, half-way through the dry season. The two dams were nearly empty and the water was turbid and unsuitable for *L. natalensis*. A search along the previous water levels did not reveal any shells of *L. natalensis*. The Athi River was examined at a point where many hundreds of cattle water daily (Plate 4.8). The river was very low at this time and two adult *L. natalensis* were found in relict pools on the river bed. They were both negative for *Fasciola*. A natural pool which still contained plenty of clear water and which had a

Plate 4.8. The Athi River. A few Lymnaea natalensis were found here in relict pools.

Plate 4.9. A natural pool where Lymnaea natalensis were plentiful.

lot of Nymphaea sp. growing in it (Plate 4.9) was also examined. Five adult L. natalensis were found in a 10 minute search. One of these contained rediae of Fasciola, another contained furcocercus cercariae, the other three being uninfected.

It was proposed to water the cattle from concrete troughs fed from a bore-hole, and this would be safe provided they were cleaned out regularly. It would seem that the two dams are potentially dangerous foci for the spread of fascioliasis as it is possible that both could become suitable habitats for L. natalensis during years with at least average rainfall. They should both be fenced off so that access for cattle is prevented at all times. The Athi River itself is not a dangerous habitat and is unlikely to become one. The river is contained between high banks and when it floods the relict pools and other possible habitats are washed away. After the floods have subsided most of the newly formed relict pools dry up before transmission can occur, although it is possible that transmission could occur in a few pools. For this reason the river should only be used for watering cattle when this is unavoidable.

The wooded drainage lines are only likely to become L. natalensis habitats in abnormally wet years and their potential danger as foci for the spread of fascioliasis can be discounted. The natural pool, where the infected L. natalensis were found, should be

fenced off.

The infectivity of very young metacercariae of F. gigantica

An experiment was carried out to assess the infectivity of very young metacercariae of F. gigantica because this point is of importance in the epidemiology of fascioliasis in relation to the possibility of infection by floating metacercariae.

The sheep (X193) used in the experiment had been at E.A.V.R.O., where natural infection with Fasciola is impossible, for six months. In the two months before the start of this experiment faecal samples taken on four separate occasions were found to be negative for Fasciola eggs.

L. natalensis were transferred to fresh water in a clean beaker and a few lengths of grass were floated on the surface. The grass was from a place where natural infection with metacercariae of F. gigantica was impossible. The beaker was put into the incubator at 26°C in the dark. After $2\frac{1}{2}$ hours it was removed, and 60 metacercariae counted out and administered to the sheep half an hour later, using techniques described in Chapter 6. The snails used in this experiment had been exposed to infection 61 days previously.

Daily collection and examination of faecal samples started 72 days after infection, and continued until F. gigantica eggs were first observed 103 days after infection. The range for 10 sheep, infected with 60

metacercariae 9 days old, (Chapter 11), was 90-105 days, with a mean of 95 days. Between 125 and 187 days after infection faecal egg counts ranged from 100 to 140 e.p.g. compared with a mean range of 467 to 704 e.p.g. in the other 10 sheep.

When X193 was slaughtered 187 days after infection, only 9 adult F. gigantica were recovered from the bile ducts and there were no immature flukes in the parenchyma of the liver. The range of lengths of these flukes was 43 to 53 mm. with a mean of 46.31 mm. This 15% recovery is much lower than that found in the other 10 sheep where the mean recovery was 62.16%, with a range of 40 to 80%.

Ten uninfected control sheep, kept with X193 and the other 10 infected ones, were all found to be uninfected at slaughter and none showed any signs of ever having been infected. All these sheep were from the same farm.

Conclusions

Metacercariae of F. gigantica less than 3 hours old are infective to sheep, but much less so than when 9 days old.

Similarly Manipol (1936), in the Philippines, was able to infect experimental animals with metacercariae of F. gigantica less than 12 hours old, but gave no details. Coyle (1961) was unable to infect several sheep and goats with large numbers of metacercariae only a few hours old, in Uganda.

Dinnik (1967, personal communication) infected a goat with F. gigantica by feeding it an infected L. natalensis. However it was impossible to repeat this experiment because of the technical difficulty of finding and removing all the metacercariae round the atrium, between the body of the snail and the shell.

Cercariae of F. hepatica could not infect mice before or immediately after their encystment, but were fully infective 24 hours after encystment according to Boray (1963).

CHAPTER 5

Infections with Fasciola spp. in African Wild Mammals

There is an increasing interest in the important role which wild ungulates could play in the development of large areas of Africa which are unsuitable, or are only marginally suited, for domestic animals (Dasmann (1964); Talbot, Payne, Ledger, Verdcourt and Talbot (1965); Beaton, Pereria, Swift, Talbot and van den Berghe (1963)).

It has become increasingly recognized (Talbot, Payne, Ledger et al. (1965)) that effective land utilization should include the use of wild ungulates. This is because the environment rapidly deteriorates with conventional animal husbandry practices, and also that these areas will maintain a substantially larger biomass of mixed wild ungulates than of domestic livestock. Wild ungulates, with their differential, non-duplicating preferred diets, more flexible water requirements and disease tolerance (such as trypanosomiasis - Cunningham (1968)), make more efficient use of the available water and forage. It should also be noted that most game meat is suitable for human consumption. There are few, if any, prejudices against eating such meat. Talbot, Payne, Ledger et al. (1965) further pointed out that it had been shown, particularly in Rhodesia and South Africa, that wild animals can be harvested and that this can be

both practical and economic. They also pointed out that it is in the potentially good pastoral land, now infested with tsetse fly (Glossina sp.), and in the semi-desert areas that game ranching could be an important new enterprise; these areas could be at least 20% of the total area of Africa.

However Attwell and Tebbit (1969) reported that in Rhodesia there are outstanding examples of successful ranching of wild ungulates, both alone and also with domestic livestock, but warned that in general the future of game ranching was in jeopardy in that country.

In addition wildlife is conserved in game parks where it can be seen and photographed by the rapidly increasing number of tourists. The tourist industry is very important in the economics of several countries in Africa. As national parks and game reserves are developed so that they can support a heavier concentration, or where they become increasingly confined and contracted, parasites may play a greater part in the dynamics of the wild animal populations. If the ranching of wild ungulates in Africa develops this will also entail restricting or preventing the seasonal migrations. It is therefore desirable to study the diseases and parasites which may become of much more importance under such artificial regimes of management.

Of more immediate importance is the effect of the

encroachment of domestic livestock on to the traditional grazing areas and watering places of wild ungulates. Where this occurs diseases and parasites are likely to be shared. Those which are harmless, or apparently harmless, to one host may be pathogenic to another. The closer and more continuous the contact between the potential hosts the more likelihood there is of such cross-infection becoming of importance.

For these reasons it was decided to carry out a survey of infections with Fasciola sp. in wild mammals, using the results together with previous reports in the literature to determine which hosts are most likely to be of importance in the epidemiology of fascioliasis.

The main questions to be studied were:-

(1) Which African wild mammals have been found naturally infected with Fasciola sp.? Are any of them likely to be pathogenic?

(2) Which of these animals are likely to be most important in the epidemiology of fascioliasis? This depends on several factors, including the frequency and intensity of infection and the longevity of the flukes in the host.

(3) Can fascioliasis persist as a disease amongst wild mammals or is the presence of domestic livestock necessary to sustain it?

(4) Is there any evidence of a new Fasciola sp., or that the known ones have adapted themselves to any wildlife species and cannot now infect domestic

animals or vice versa?

Methods

To obtain the data on which a consideration of these questions could be based the co-operation of veterinarians and zoologists with access to autopsy findings on wild mammals was sought. The following information was asked for:-

(1) Species of host examined (scientific names).
(2) Approximate age of host.
(3) Sex of host.
(4) Geographical location.
(5) Number of hosts examined.
(6) Number of flukes recovered.
(7) Frequency of contact between host and domestic ruminants.
(8) Description of watering places used by hosts.

Instructions were also issued which detailed the collection and preservation of the liver flukes.

Results

The recorded hosts of Fasciola gigantica in African wildlife are in Table 5.1. The results of the survey are in Table 5.2. All the liver flukes from African wild mammals were considered to be F. gigantica, except for one record of Fasciola nyanzae. The records of F. gigantica infection in wildlife in Africa reported by previous authors are shown in Table 5.3, while Table 5.4 shows reports of Fasciola hepatica and

Table 5.5 of Fasciola (species unknown). Tables 5.6 and 5.7 are reports of F. nyanzae and Fasciola tragelaphi respectively.

Table 5.1

Recorded hosts of Fasciola gigantica in African wild mammals

	Species	No. of records
Order Proboscidea		
Family Elephantidae	Loxodonta africana	1
Order Perissodactyla		
Family Equidae	Equus sp.	(4)
Order Artiodactyla		
Family Hippopotamidae	Hippopotamus amphibius	3
Family Giraffidae	Giraffa camelopardalis	3
Family Bovidae		
Sub-family Cephalophinae	Sylvicapra grimmia	2
Sub-family Antilopinae	Aepyceros melampus	1
Sub-family Hippotraginae	Connochaetes taurinus	7
	Alcelaphus buselaphus	2
	Kobus defassa	1
	Kobus varondi	1
	Damaliscus korrigum	2
	Redunca arundinum	1
	Kobus kob	5
Sub-family Bovinae	Syncerus caffer	7
	Taurotragus oryx	4
	Tragelaphus strepsiceros	1

Of a total of 45 records in African wild mammals, 34 are in the family Bovidae, and 31 of these are in the two sub-families Hippotraginae (19) and Bovinae (12).

Table 5.2

Survey results - Fasciola gigantica in African wildlife

Host species	Location	Contact with domestic ruminants	No. of animals examined	Per cent infected	Largest burden of F.gigantica recorded	Source
Syncerus caffer (Sparrman, 1779) African buffalo	Aswa/Lolim Game Reserve, Uganda	Rare or nil.	5	100	17	Royal Vet. College, East Afr. Res. Team, 1968
	"	"	82	52.8	66	Bindernagel (1969, pers. comm.)
	Serengeti National Park, Tanzania	Rare	10	20	5	Sachs (1969, pers. comm.)
	Queen Elizabeth Nat.Park, Uganda	Probably never	46	2.2	-	Bindernagel (1969, pers. comm.)
Kobus kob (Erxleben, 1777) Kob, includes Uganda kob.	Aswa/Lolim Game Reserve, Uganda	Probably never	103	46.5	17	Bindernagel (1969, pers. comm.)
	"	"	2	50	15	Sachs (1969, pers. comm.)
Redunca arundinum (Boddaert, 1785) Reedbuck	Rhodesia	-	2	-	-	Condy (1970, pers. comm.)
Giraffa camelopardalis (Linnaeus, 1758) Giraffe	Kenya (Edinburgh Zoo)	-	1	-	41	Hammond (1966, unpublished).
Damaliscus korrigum (Ogilby, 1837) Topi	Serengeti National Park, Tanzania	Seasonal/ continuous	105	3.8	30	Sachs. (1969, pers. comm.)
Connochaetes taurinus (Burchell, 1823) Wildebeest	Nairobi Nat. Park, Kenya	-	-	-	-	Collection J.A.Dinnik (1961)
	Kajiado, Kenya	-	-	-	-	Collection J.A.Dinnik (1961)
	Serengeti Nat.Park, Tanzania	Seasonal/ continuous	90	6.6	7	Sachs (1969, pers. comm.)
	Voi, Kenya	-	1	100	1	Hammond (1967, unpublished)
Alcelaphus buselaphus (Pallas, 1766) Kongoni	Aswa/Lolim Game Reserve, Uganda	Probably never	47	46.8	21	Bindernagel (1969, pers. comm.)
Sylvicapra grimmia (Linnaeus, 1758) Grey duiker	Rhodesia	-	4	100	-	Condy (1970, pers.comm.)
Taurotragus oryx (Pallas, 1766) Eland	Nairobi Nat.Park, Kenya	-	-	-	-	Collection J.A.Dinnik (1961)
	Serengeti Nat.Park, Tanzania	Rarely/ seasonal	12	8.3	1	Sachs (1969, pers. comm.)
Aepyceros melampus (Lichtenstein, 1812) Impala	Nanyuki, Kenya	-	1	100	Approximately 7	Clausen, (1968, pers. comm.)

Comments: Condy (1970, pers. comm.) reported 2 deaths in the reedbuck and 4 deaths in the grey duiker in Rhodesia. Hammond (1966, unpublished) found 41 live F. gigantica in a giraffe which died in Edinburgh Zoo 5 years and 56 days after arriving in the United Kingdom from Kenya.

Table 5.3

Previous reports of Fasciola gigantica in African wildlife

Host species	Country	Authors comments	Reference
Syncerus caffer	The Sudan		Myers, Wolfgang and Kuntz (1960)
	Uganda	No contact with domestic ruminants within living memory	Coyle (1961)
	Central African Republic	1 infected out of the 5 which were examined	Graber, Doutre, Finelle, Keravec, Ducroz and Mokotainger (1964)
Kobus varondi (Livingstone, 1857) Puku	Zambia	One immature F. gigantica recovered	LeRoux (1939)
Kobus kob	Uganda	-	Dinnik (1963)
	The Congo	-	Stunkard (1929)
	Chad	1 infected out of the 6 examined	Graber, Doutre, Finelle et al (1964)
	Chad	-	Graber (1969)
Kobus defassa (Ruppell, 1835) Defassa waterbuck	The Congo	-	Stunkard (1929)
	Chad	-	Graber (1969)
Hippopotamus amphibius Linnaeus, 1758 Hippopotamus	Zambia	-	LeRoux (1934)
	Not stated	-	LeRoux (1955)
Giraffa camel-opardalis	Ethiopia	"no fewer than 40"	Cobbold (1855)
	Not stated	F. gigantica-type eggs in faeces	Porter (1943)
Damaliscus korrigum	The Sudan	-	Myers, Wolfgang and Kuntz (1960)
Connochaetes taurinus	Kenya	-	Urquhart, Hay, Zaphiro and Spinage (1960)
	Western Masailand, East Africa	1 infected out of 30 calves "occasionally in adults"	Talbot and Talbot (1963)
	Rhodesia	Reported deaths from this infection	Lees May (1967)
Alcelaphus buselaphus	The Congo	-	Stunkard (1929)
	Chad	-	Graber (1969)
Sylvicapra grimmia	Rwanda	-	Fain (1951)
Taurotragus oryx	Uganda	-	Coyle (1961)
	Rhodesia	Reported deaths from this infection	Lees May (1967)
Loxodonta africana (Blumenbach, 1797) African elephant	Uganda	-	Coyle (1961)
Tragelaphus strepsiceros (Pallas, 1766) Kudu	Rhodesia	Reported deaths from this infection	Lees May (1967)

Comments (Table 5.3): Coyle (1961) reported finding liver flukes similar to F. gigantica in the hippopotamus and the elephant in Uganda.

Cobbold (1855) gave the first description of F. gigantica. In an earlier publication (Cobbold, 1854) it was stated that the host animal had been imported from Abyssinia. In his 1860 publication Cobbold again referred to his original finding. One reason for the very few records from the giraffe, the type host, is that in many countries there are severe restrictions on the numbers which are allowed to be hunted.

Sachs and Sachs (1968) reported that buffalo, eland, wildebeest and topi infrequently harboured a small number of F. gigantica in the Serengeti Region. They only examined the livers in their survey as at routine meat inspection and so their figures for the numbers of liver flukes recovered must be regarded as the minimum in each case.

Porter (1938) stated that F. gigantica occurred in antelope in South Africa but Neitz (1965) reported that neither F. hepatica nor F. gigantica had been found in wildlife there.

Porter (1920, 1938), Pilsbry and Bequaert (1927) and Brumpt (1936) all reported that F. gigantica had been found in the "zebra" but gave no references to the sources of their information. It is probable that they all referred to the one source, and they also, in

the same context, referred to the infection in giraffes and buffaloes and these references are not included in the tables either.

Table 5.4

Reports of Fasciola hepatica in African wild mammals

Host species	Country	Authors comments	Reference
Alcelaphus sp.	The Congo	-	Stunkard (1929)
Kobus sp.	The Congo	-	Stunkard (1929)
Syncerus caffer	The Congo	In the Uturi forest	Sandground (1929)
	The Congo	In the Uturi forest	Sandground (1930)
	The Congo	In the Uturi forest	Strong and Shattuck (1930)

Comments: Stunkard (1929) stated that he found F. hepatica in the livers of Kobus, Adenota and Alcelaphus. As there is no Adenota species this is included as one record under Kobus sp. and one under Alcelaphus sp. However it is possible that he was referring to Kobus defassa and Adenota (=Kobus) kob, as he did under F. gigantica in the same paper.

Table 5.5

Reports of Fasciola (species unknown) in African wild mammals

Host species	Country	Authors comments	Reference
Hippopotamus amphibius	Zambia	-	LeRoux (1957)
Kobus leche Gray, 1850 Lechwe	Zambia	-	LeRoux (1957)
Loxodonta africana	Uganda	Eggs of a Fasciola sp. in faeces	Dinnik, Walker, Barnett and Brocklesby (1963)

Comment: LeRoux (1957) stated that these were "liver fluke" infections. It has been assumed that they were Fasciola sp.

Table 5.6

Reports of Fasciola nyanzae in Hippopotamus amphibius

Country	Authors comments	Reference
Uganda	-	Leiper, 1910
East Africa	-	Jackson, 1921
Uganda	-	Dinnik and Dinnik (1961)
Uganda	Most of the 287 examined were infected	Guilbride, Coyle, McAnulty, Barber and Lomax (1962)
Uganda	9 of the 15 examined were infected	Dinnik, Walker, Barnett and Brocklesby (1963)
South Africa	94 out of the 97 examined were infected, i.e. all except infants	McCully, van Niekerk and Kruger (1967)
Uganda	-	Royal Vet. College, E.A. Res. Team Interim Report, 1968
Rhodesia	A common infection	Condy (1970, pers. comm.)

Comments: There are no records of this parasite in any other animal. Coyle (1961) was unable to find it in cattle in Uganda. Furthermore, Dinnik and Dinnik (1961) were unable to infect 3 calves, 3 goats and 5 rabbits.

McCully, van Niekerk and Kruger (1967) also studied the pathology of this infection.

Table 5.7

Reports of Fasciola tragelaphi in Tragelaphus spekei Sclater, 1864; Sitatunga

Country	Authors comments	Reference
Rhodesia	-	Pike and Condy, 1966
Rhodesia	3 infected animals reported	Condy (1970, pers. comm.)
Uganda	-	Pullan (1966, pers. comm.)
Uganda	-	Bwangamoi (1968)

Comment: This parasite has only been reported in the sitatunga.

Discussion

It is not surprising that F. gigantica accounts for most infections in wild mammals as it is the commonest liver fluke in domestic animals over most of Africa. Both F. nyanzae and F. tragelaphi have each only been reported from their type hosts - it is possible that they may be found to be infective for a wider spectrum of animals. However Condy (1970, personal communication) had not found kudu and bushbuck (Tragalaphus scriptus (Pallas, 1766)), two species which are closely associated with the sitatunga, to be infected.

The fact that infections with Fasciola sp. have not been recorded in some wildlife species does not

necessarily mean that they cannot be infected with these parasites; it may be that the species are only rarely exposed to infection because of their grazing or browsing habits, or because they rarely or never visit watering places. The natural history of wild mammals is discussed by Talbot and Talbot (1963), Dasmann (1964), Morris (1965) and Lamprey (1963, 1964). However under altered and more intensive systems of range management they might be exposed more often and might then become important in the epidemiology of the disease. It would therefore be desirable to infect experimentally some of those species of animals which have never been recorded in the literature as being found infected, but which could become important in ranching schemes, e.g. Thomson's gazelle (Gazella thomsonii Günther, 1884), and to study the pathogenesis and course of the disease in such hosts.

The wild mammals which have to date been found naturally infected with Fasciola sp. are listed in the tables. Most of the reports are from Syncerus caffer (the buffalo), Connochaetes taurinus (the wildebeest), Kobus kob (Uganda kob), Taurotragus oryx (the eland), Giraffa camelopardalis (the giraffe) and Hippopotamus amphibius (the hippopotamus). These are followed by Alcelaphus buselaphus (the kongoni), Damaliscus korrigum (the topi) and Sylvicapra grimmia (the grey duiker) with two records each. The other host species have been found infected on one occasion only.

However, there is not enough data to present this confidently as a true picture of the relative importance of the various potential hosts. Furthermore many more animals of some species have been examined than of others, so that the samples are accordingly biased.

There are some notable exceptions to the table (Table 5.1) of the recorded hosts of F. gigantica. There have been only two records in animals belonging to the sub-family Cephalophinae (the duikers), and only one in those of the sub-family Antilopinae, that is, in the impala. The latter sub-family includes several common species such as Thomson's gazelle, Grant's gazelle (Gazella granti Brooke, 1872) and the oribi (Ourebia ourebi (Zimmerman, 1783)). Bindernagal (1969, personal communication) examined the livers of 20 of the latter species in the Aswa/Lolim Game Reserve in Uganda and none were infected - he has reported a high percentage of other species of animals to be infected in the same area (Table 5.2). According to Morris (1965) this is a grazing species living in grassland generally within easy reach of water, and so might be exposed to infection. However the sample is too small to be able to draw any valid conclusions. It is less surprising that no member of the family Suidae has been found naturally infected, for it is known that the pig is very resistant to infection with F. hepatica (Ross, Dow and Todd, 1967).

However, this may not be so marked with F. gigantica for in the Hawaiian Islands wild pigs were reported to be naturally infected (Alicata and Bonnet, 1956), and Alicata (1938) was able to infect a pig experimentally and reported a recovery rate of 27% when the animal was slaughtered 64 days after infection. Furthermore the flukes were between 20 and 28 millimetres long at this age. The Royal Veterinary College, East Africa Research Team (1968, personal communication) examined 23 wart hogs (Phacochoerus aethiopicus (Pallas, 1766)) in the Queen Elizabeth National Park in Uganda and all were uninfected. This again is too small a sample from which to be able to draw valid conclusions, also the incidence of F. gigantica infection in animals known to be susceptible is very low in this Park (Table 5.2, Bindernagel, 1969, personal communication). No evidence of the longevity of F. gigantica could be obtained from the survey as such. However, at the post-mortem examination of a giraffe which died in Edinburgh Zoo in 1966, 41 live specimens were recovered. This animal had arrived in the United Kingdom 5 years and 56 days before death. Infection with this parasite in Edinburgh is considered to have been most unlikely in the absence of an intermediate snail host. Natural infection has never been recorded in Britain and the giraffe was fed entirely on a local diet. Thus it would appear that F. gigantica can live for at least this period in the

giraffe, and it is therefore possible that this host is of some importance in the spread and maintenance of foci of infection.

Coyle (1961) recovered liver flukes, which he believed to be F. gigantica, from elephant and buffalo in Uganda from an area in which there had been no domestic livestock within living memory, and because of this he suggested that this fluke may be indigenous to Africa. Also Bindernagel (1969, personal communication) found a high percentage of the buffalo, Uganda kob and kongoni infected in the Aswa/Lolim Game Reserve in Uganda, where there was almost certainly no contact with domestic ruminants, except that some buffalo might at times graze land which was also grazed by zebu cattle, but the other species did not. This Game Reserve was an important cattle area until the early years of this century, when human sleeping sickness caused it to be evacuated by both people and stock and only then did it become an important game area. It would thus appear that fascioliasis can be maintained among some species of game animals and that domestic livestock are not necessary.

There was no indication in this survey of any previously undescribed species of Fasciola, although the specimens received during the survey from some host species were larger than those from others. Nor was there any evidence that the known Fasciola sp. have so adapted themselves to any wildlife species that they

cannot now infect domestic animals. This latter point can only be conclusively proved following experimental infections, although a larger survey in selected areas would provide further indicative evidence.

In view of the large number of wild ungulates that have now been shown to be susceptible to infection with F. gigantica, the widespread incidence of this parasite in domestic livestock over a large part of Africa, and the absence of evidence that it cannot spread from one species to another, measures should be taken to prevent or at least limit the spread of this parasite when new ranching schemes are formulated. This would involve measures to reduce or eliminate contact between infected faeces and Lymnaea natalensis. Condy (1963), referring to the Wankie National Park in Rhodesia, suggested that the watering places used by wild ungulates should be allowed to dry out completely, or that molluscicides should be used, to kill the host snails. It would seem that under natural conditions many watering places used by wild ungulates are not likely to be suitable habitats for L. natalensis because they do dry out every year. If, however, they are kept filled by artificial means or converted into permanent water holes, they could then become suitable habitats. Even where suitable habitats are present wildlife which follows an annual migratory pattern is likely to be less exposed to infection than one which

does not do so. This is because snails which become infected at one visit may largely have died off before the next one, especially as water levels may have altered considerably in this time, and those snails which are in shallow water and which are the more likely to become infected are also the more likely to die of desiccation. Further, metacercariae may have also died of desiccation before the next visit.

LeRoux (1955) stressed the desirability of snail control in Africa if the fauna in game reserves was to be preserved, and considered that the arrest of the seasonal movements of game meant that the animals were continuously exposed to severe re-infections. He thought that the building of earth dams for the conservation of water to arrest migration provided ideal habitats for snails.

Fasciola hepatica in Europe naturally infects the rabbit (Oryctolagus cuniculus) which is a very important reservoir host (Taylor, 1964). It is likely that a similar state of affairs could exist in Africa with small indigenous animals. Coyle (1961) reported that rabbits were numerous in Africa but were Lepus capensis, a different species from that common in Europe. He shot 50 of these in an area in Uganda where F. gigantica was common in livestock and found only one infected, with one liver fluke - this was not positively identified as a Fasciola sp., but, as it was 10 millimetres long while still in the liver parenchyma

it is likely that it was. In Rhodesia, Condy (1962) reported that domestic bovines were the main carriers of F. gigantica, and that rabbits did not occur. As there is no evidence from the small indigenous animals of Africa, experimental infections of the most likely hosts would seem to be indicated. The finding that one of these could also be used as a suitable host in the laboratory would be an additional benefit.

The reports from Rhodesia (Condy, 1970, personal communication; Lees May, 1967) of game animals dying from F. gigantica infection are the first that have been recorded (Tables 5.2 and 5.3). So far five species have been listed. Insufficient information is available to assess the reason for these deaths which occurred in the Lake McIlwaine Game Park and on a farm.

Conclusions

1. Fascioliasis is not very prevalent in wild mammals except for F. nyanzae in the hippopotamus.

2. There is some evidence from Uganda that F. gigantica infection can be maintained in wildlife in the absence of domestic ruminants.

3. There are signs that it is becoming of more importance, and deaths have been reported from Rhodesia in five species. It is not known how much these are due to the developments forecast earlier or to abnormally wet seasons.

4. Although no small animal hosts have been found, it would seem that the giraffe at least could be an efficient reservoir host.

SECTION III

Experimental materials and methods

Chapter 6 The production of infective material

Chapter 7 Laboratory materials and methods

Chapter 8 Experimental animals

CHAPTER 6

The Production of Infective Material

(A) Production of Fasciola hepatica metacercariae

Taylor and Mozley (1948) developed a satisfactory method of breeding Lymnaea truncatula by reproducing the natural habitat in glass and earthenware vessels. Their method was modified by Sewell (1962).

L. truncatula was already available from stock colonies in Edinburgh, and the methods used for their culture, the collection and hatching of F. hepatica eggs, infection of the snails and collection of metacercariae were essentially those described by Sewell (1962). They have also been described in detail, with modifications for large-scale production of metacercariae, by Pullan (1968), and therefore only some personal observations are recorded.

These methods proved very satisfactory and no serious difficulties were encountered. It was found satisfactory to store prepared algal cultures, which had been hermetically sealed, in the dark in the cold room at 8-10°C for some weeks. Kendall and Parfitt (1965) stored similar cultures at -4°C for some months. However, in one case all snails feeding on a thick, dark green algal culture, which had been stored under these conditions, died after a period of two days. The rest of the same batch of cultures was discarded as it was considered that a toxin may have been

responsible for the deaths. This incident shows the advisability of using small snail units so that these accidents are the more likely to be confined to one small part of the whole, and the same principle was used with the culture of Lymnaea natalensis below.

The problem of fungal growth on the cultures was essentially a winter problem, and was overcome by keeping the cultures in the hot room at 37°C and by achieving a quick primary cover of algae. This seeding technique was carried out by using washings from mature cultures to moisten new ones.

Management of snails in the laboratory

The L. truncatula colonies thrived on the algal cultures when kept at about 23°C in the incubator. They were examined daily when any dead snails were removed. Faeces and, when present, the thick mucus secreted by the snails when they were in the cold water which was used to stimulate cercarial emission, was washed off at regular intervals with distilled water. Standen (1963) stated that L. truncatula is an amphibious snail that spends most of its life out of water but will not thrive if denied access to it. Here, however, it thrived on a moist surface in a saturated atmosphere.

Pullan (1968) referred to the difficulty of transferring very small snails to fresh cultures without damaging them. However, the following technique was found to be very satisfactory. The

snails were washed off the old culture with a jet of distilled water from a plastic wash-bottle into a white tray. After a few minutes the water was gently agitated and decanted into a second tray. More distilled water was used to wash off all the mud from the first tray and again poured off into the second one. Most of the young snails were left attached to the bottom of the first tray and were transferred to the new algal culture with a camel-hair brush. The process was repeated with the second tray.

Techniques used with metacercariae

At first the metacercariae, which were attached to the inside of small polythene bags, were stored under water inside 4 oz. screw-capped bottles kept in the dark at about 10°C. Later, after Boray and Enigk (1964) had found that metacercariae stored at 10°C and a relative humidity (RH) of 90% were viable for at least 122 days, a wet piece of cotton wool was placed at the bottom of each bag to keep the sides apart. These were then stored as previously described. This technique ensured a saturated atmosphere and a higher oxygen tension for the metacercariae than when deep water was used.

For the acute fascioliasis experiment, where large numbers of metacercariae were required, the method used for counting the infective doses was that described by Pullan (1968). For the chronic fascioliasis experiment, the cysts were counted and handled while

still adherent to the polythene, a method similar to that described by Urquhart (1954). The polythene bags were cut up into strips, and the strips were marked with a mounted needle at a width that nearly filled the field of the stereomicroscope used to count the cysts. Each strip was placed cyst-side downwards on a microscope slide which was kept moist all the time. The viable cysts were then counted, a suitable magnification being used so that apparently normal cysts could be identified, and only these were counted. If there was any doubt as to the viability of a cyst it was removed.

After counting the cysts the polythene strips were stored as described previously, the infective dose for each animal being kept in separate bottles. Several sheets of polythene had to be used with each animal and, in order to ensure that these separated so that the excysted flukes could escape, the sheets were interleaved with damp filter paper before being rolled up and administered to the sheep with a balling gun.

When the stage of the stereomicroscope became warm, because of the electric light bulb under it, some of the metacercariae were activated and were seen to move inside the cyst. Steps were taken to minimise the risk of this activation occurring by reducing the temperature as much as possible, as Sewell (1962) had observed that if the temperature of the cyst's environment is allowed to fall after such activation

the metacercariae cease to move and become refractory to re-activation. In view of the very low recovery rate of flukes from the livers of the infected animals, the range was 1.6% to 15.3% of the metacercariae which had been administered, it is thought that a much higher proportion of metacercariae may have been activated than was apparent at the time. However, Boray and Enigk (1964) also found that microscopical examination alone is not sufficient as a criterion of viability. Further, excystation *in vitro* only gives evidence of viability and not of ability to invade the liver and reach maturity.

(B) *Production of Fasciola gigantica metacercariae*

Kendall (1965) considered that all the snail hosts of *F. gigantica* belong to the superspecies of *Lymnaea auricularia*, having very similar ecological requirements. The natural habitats of *Lymnaea natalensis* the intermediate snail host in Africa have been considered by Hammond (1965).

Several authors have described methods for maintaining cultures of the superspecies *L. auricularia* in the laboratory (Porter, 1920; Alicata, 1938; Kendall and Parfitt, 1953; Dinnik and Dinnik, 1955; Ghani, 1960; Coyle, 1961; Taylor, 1961; Standen, 1963; Guralp, Ozcan and Simms, 1964; Kendall and Parfitt, 1965; Rao, 1966; Boray, 1963, 1966 and Bitakaramire, 1968).

The details of the methods used vary greatly.

Only Bitakaramire (1968), Boray (1966), Standen (1963) and Ghani (1960) maintained balanced systems, but Coyle (1961) found that such a system was very difficult to attain in the laboratory. In the system developed by Boray (1966) the water was re-circulated through a Perlon-wool filter. The balanced type of aquarium used by Ghani (1960) consisted of a bath tub of 200 litres capacity in which the natural habitat of the snail was reproduced.

Most authors used a food supplement such as lettuce, or an artificial food-mixture such as calcium alginate gel (Kendall and Parfitt, 1965; Rao, 1966). Standen (1963) also fed an alginate food, and Taylor (1961) sprinkled a calcium supplement of powdered egg shells on the water twice a week, while Boray (1966) used 2-3% calcium sulphate mixed with the food.

The importance of aeration of the water in the culture was stressed by many workers. This was sometimes achieved by the use of aquatic plants to provide natural aeration, usually combined with shallow water in the culture vessels, i.e. a large surface area-to-volume of water. Such methods of aeration were employed by Taylor (1961) and Standen (1963). However other workers used a pump to provide forced aeration (Boray, 1966; Bitakaramire, 1968).

Dinnik and Dinnik (1964) showed that at temperatures of 16°C or less the rediae did not produce cercariae, but when the temperature rose to a mean

maximum of 20°C all the rediae changed from redial to cercarial production. Thus a temperature of over 20°C must be maintained if metacercariae are required. Boray (1966) maintained his cultures at 22-24°C while Bitakaramire (1968) and Coyle (1961) kept theirs at 26°C. Dinnik and Dinnik (1963) further discussed the effect of seasonal variations of temperature, under laboratory conditions, on the development of F. gigantica in the snail host in the Kenya highlands.

Nearly all authors stressed the importance of having clean water in the culture vessels at all times, and in those systems where balanced conditions were not attempted frequent changes of water were necessary. This varied from nearly every day (Rao, 1966) to 5 days (Taylor, 1961) and even 7 days (Alicata, 1938), but 2-3 days seemed to be the average time thought necessary by most workers.

Whereas Boray (1963, 1966) used de-ionised water, most other authors found that untreated water from ponds, streams or wells was satisfactory (Bitakaramire, 1968; Alicata, 1938; Taylor, 1961). However, Kendall and Parfitt (1965), Guralp, Ozcan and Simms (1964), Standen (1963) and Ghani (1960) used tap water for their culture vessels, and this was after previous chlorination in the latter case.

The growth of an algae (species not stated) was mentioned by Taylor (1961) as an occasional hazard which, if profuse, colonises and kills the eggs and

smothers the adult snails. Coyle (1961) discussed in considerable detail the problems he had when trying to maintain colonies of infected L. natalensis. He found that some of the blue-green algae of the Class Myxophyceae appear harmful, but that the establishment of suitable algal species seems to be encouraged by vigorous aeration and frequent changes of water. High mortality of the snails was found by Standen (1963) to be caused by excessive growth of filamentous algae, a condition he considered more likely to occur in the balanced type of aquarium subjected to over-illumination.

The methods described by Kendall and Parfitt (1953, 1965) were developed from the algal mud-slope technique of Taylor and Mozley (1948) for use with L. truncatula. These authors found (1953) that Lymnaea auricularia rufescens readily accommodated itself after the addition of one or two inches of water in the culture dishes. Later (1965) this technique was developed for the culture of other vector snails of F. gigantica, including L. natalensis. Also Boray (1963) found that his shallow-water system for the continuous breeding of Lymnaea tomentosa was suitable for L. natalensis. He also noted that with L. truncatula after several generations an adapted strain emerged, the average size of the snails increased and a lower mortality was found after infections. Similar observations have been made by

Dinnik (cited by Boray, 1963) with L. natalensis.

Management of snails in the laboratory

The colonies of L. natalensis were already available at E.A.V.R.O. where they had been established some years before. The snails had been obtained from a local natural habitat and the methods in use, which were developed by Dr. and Mrs. Dinnik, have not been adequately described. It was a modification of these methods which was used to produce the large numbers of viable metacercariae needed for this work and this will be described.

The breeding colonies of L. natalensis were maintained in enamelled metal bowls of 48-53 cm. outside diameter, 12½-15 cm. depth and containing 8-10 litres of water. Plates 6.1 and 6.2 show cultures of L. natalensis. When it was necessary to have as many breeding colonies as possible, glass containers 25 cm. x 25 cm. x 30 cm. deep containing about 12 litres of water were also used. In all types of container the normal level at which the water was maintained was 4-5 cm. from the top of the vessel. About 1" of well-washed local sand was put into the bottom of each aquarium. White coral sand was perhaps better as the snails could be seen more easily, and it may also have served as a source of calcium for the snails, although there was no evidence that this was too low for satisfactory shell formation when using the local sand.

Aquatic plants were kept in all the aquaria to

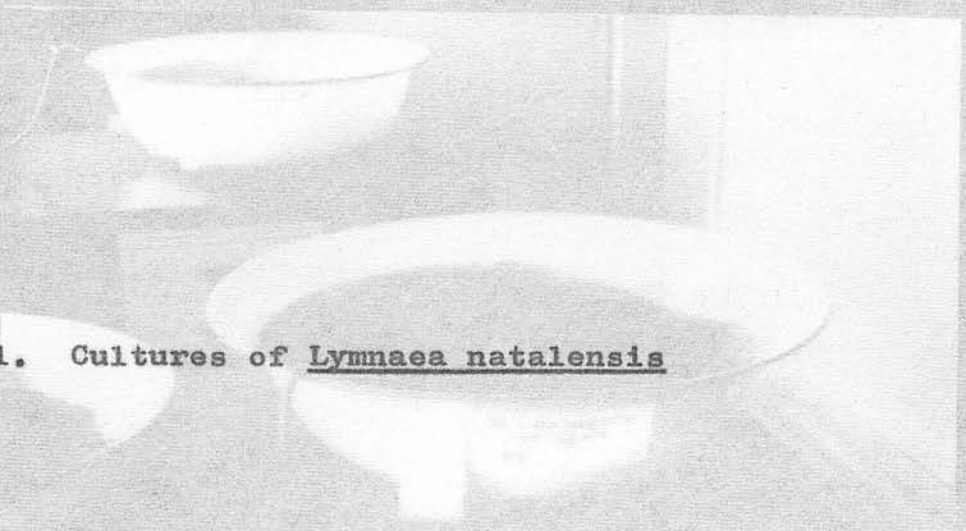


Plate 6.1. Cultures of Lymnaea natalensis

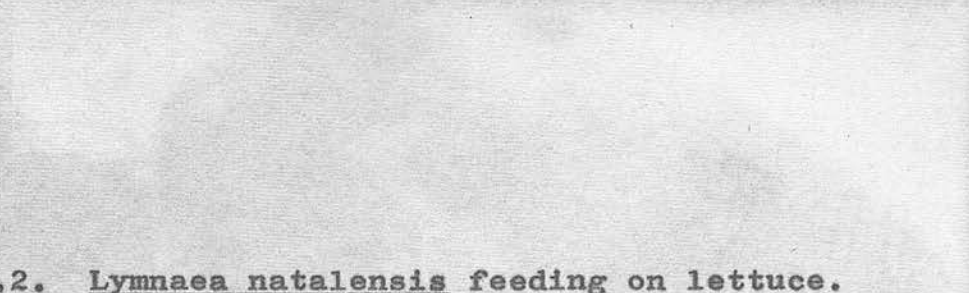


Plate 6.2. Lymnaea natalensis feeding on lettuce.


② L. NATALENSIS.
INF. 10½ NOV.
24 B

increase the surface area available for algal growth for the snails to feed on, also for the deposit of egg masses and to aid in the aeration of the water. Nymphaea sp. (probably N. caerulea Savigny) was especially favoured by the snails for depositing egg masses on, but other local aquatic plants, which were commonly used as well, were Utricularia sp. (probably U. inflexa Forsh), Elodea densa (Planch) Casp., and Nitella sp. (? N. oligospyra Br.). These aquatic plants were identified by Mr. J. B. Gillett. Pieces of rock or coral were placed in the centre of the bowl to help to anchor the aquatic plants which were planted in the sand.

All the water used in the aquaria was collected in galvanized iron or aluminium churns once a week, from a local stream which was known to be a L. natalensis habitat. This water was stored in the open churns until used. It was always left for two days after collection because of the possible danger to the laboratory staff of infection with Schistosoma sp. The possibility of contamination of the water supply with agricultural or veterinary pesticides, and especially those containing copper ions, was considered and an experiment was carried out using tap water, pumped from a bore-hole, in one aquarium instead of the stream water. In the 16 months during which this was done there were no apparent ill effects on the rate of breeding, growth or mortality and shell formation was

also satisfactory.

Over a 3 month period the pH of the aquaria varied between 6.8 and 8.2. There was a tendency for it to become more alkaline by the time the aquaria required cleaning out.

The snails fed on the algae which multiplied rapidly in the aquaria, but boiled lettuce was also given as a supplementary food every day. For the larger snails the fresh lettuce was boiled for 5 minutes and strips were then laid on the surface of the water. The aim was to give only the amount that would be just consumed before the next feeding. The lettuce for the young snails was boiled for 15 minutes, then squeezed through wire mosquito gauze of about 2 mm. square aperture. The resultant mulch was well dispersed among the snails. Every day each aquarium was examined and dead snails, unconsumed lettuce or other detritus was removed with the aid of forceps and a small nylon strainer.

No attempt was made to maintain a balanced ecosystem in the aquaria so they all had to be cleaned out at 1 to 4 weekly intervals, with a mean of $2\frac{1}{2}$ weeks. The time between successive cleanings depended on many factors and especially on the number and size of the snails in each aquarium. The decision to clean out an aquarium depended on the amount of snail faeces and other detritus present, the appearance of the water, an increase in the snail mortality or a tendency

for the snails to move to the edge of the water or to leave the aquarium altogether.

Each aquarium to be cleaned was removed to the sink where all the plants and attached snails were removed, put into a tray and covered with water. Other visible snails were then removed with a nylon strainer, a camel-hair brush or special metal forceps which were made from very thin steel and which could not damage the snails, and put into a beaker of water. The water was then poured out of the aquarium through a nylon strainer which retained any snails remaining in the water. Snails on the surface of the sand were removed before the sand was well washed with at least four changes of tap water, and one of the natural habitat water, before being refilled with fresh water. The aquatic plants were washed lightly, to remove faecal matter, before being returned to the basins with all the snails. A record was kept of the occasions when the basins were cleaned.

The aquatic plants had to be renewed at long intervals. These were collected from a local habitat of L. natalensis, and left for a week in each of four glass tanks before being used in the culture basins. This was done to avoid contamination with other species of snails or their eggs.

At first, in order to ensure optimal oxygen tension, air was bubbled through the water continuously, using "Hyflo" Model B pumps (Medcalf

Bros.). However the large surface area in relation to the volume of water in the enamel basins appeared to ensure that oxygenation was sufficient without this, so this forced aeration was discontinued except in the case of those aquaria which contained infected snails. It was the general practice to move infected snails into smaller glass tanks shortly before the collection of metacercariae started. This was to facilitate their collection for the shedding of cercariae.

The breeding colonies of snails were usually maintained in a separate house from the infected ones. The former were kept at between a daily mean minimum air temperature of 13.5°C and a daily mean maximum of 19°C, at the coldest time of the year in July. The infected snails were maintained at 24-28°C with no seasonal variation. If many young snails were needed, more quickly breeding colonies were also kept at the higher temperature. About 30 L. natalensis of 8 mm. or more in length were kept in each basin, together with numerous small ones.

An indication of the suitability of the management can be obtained from the number of eggs found in the egg masses. On one occasion at the coldest time of the year 31 egg masses were taken at random. These contained 6-27 developing embryos in each, with a mean of 15 per mass. On a later occasion, when the temperature was slightly higher, two further samplings were carried out. One collection of

30 egg masses had a range of 6-60 developing embryos with a mean of 31 per mass, and the other collection of 16 egg masses had a range of 14-41 developing embryos per mass with a mean of 25 per mass. Bitakaramire (1968) stated that as many as 100 snail embryos were counted in an egg mass 40 mm. long, but gave no ranges or means, and Kendall and Parfitt (1965) reported that the greatest production of eggs occurred in the earlier part of the egg-laying period and that initially as many as 25 eggs might be found in a single egg mass. Snails of different age groups were included in each aquarium used for breeding at E.A.V.R.O., but the results would seem to compare favourably with these. Taylor (1961) recorded a range of 12-68 embryos in each egg mass but he gave no means, and neither did Coyle (1961) who did however give a range of 20-50. Ghani (1960) gave a mean of 13.8 embryos per egg mass with a maximum of 140, and Alicata (1938) a range of 1-92.

Chaetogaster limnaei (Baer)

Very early in this work it was observed that many L. natalensis were harbouring a parasite that appeared to be Chaetogaster limnaei (Gruffydd, 1965a, 1965b). Infested snails were sent to Dr. Ll. D. Gruffydd who identified these oligochaetes, on the basis of their external morphology, as Chaetogaster limnaei limnaei. Although these are not pathogenic for the snails there have been reports from the literature that they ingest

miracidia. Both Khalil (1961) and Coyle (1961) observed miracidia of F. gigantica in the gut of Ch. limnaei, and Michelson (1957) found that Ch. limnaei swallowed miracidia and cercariae of schistosomes. Khalil (1961) was not able to infect any of 500 L. natalensis of all ages which were infested with Ch. limnaei with 5-10 miracidia of F. gigantica, and Coyle (1961) conducted a series of experiments, with three separate lots of infested L. natalensis, which he exposed to 10, 20 and 100 miracidia per snail respectively. None of the snails in the first two lots became infected, and only 60% of the group which were exposed to over 100 miracidia each were infected. Coyle used snails naturally infested with about five Ch. limnaei each for the first experiment, but these numbers had increased to from 15-30 per snail for the last experiment. Similarly Michelson (1964) found that infestation with Ch. limnaei afforded a degree of protection to Australorbis glabratus when it was exposed to miracidia of Schistosoma mansoni, and to a lesser extent they protected snails exposed to Echinostome miracidia.

It is of course possible that other factors were interfering with the infection process, but the evidence of the effect of Ch. limnaei infestation is strong. Hence it was necessary to find a means of obtaining L. natalensis free, or almost free, of infestation with Ch. limnaei. One way would have been

to start with fresh aquaria, and egg masses of L. natalensis free of these oligochaetes. As Chaetogaster probably occurs in the natural habitats of L. natalensis, the water from such a habitat being used in the aquaria at that time would have had to be passed through a very fine filter before use and problems would also have arisen concerning the sterilization of the aquatic plants. It was also observed that a low infestation of Ch. limnaei can very rapidly build up to high levels under laboratory conditions at E.A.V.R.O., so that any faulty or careless technique would have serious consequences. Accordingly this method was not considered practical at that time.

Michelson (1964) had recommended the use of 1% Urethan (ethyl carbamate) to remove the parasite from the snails. This method had been tried by Dinnik with little success (personal communication, 1967). However Dinnik had obtained good results by heat treating the infested snails, and from this the following technique was developed. A large beaker of water was heated to 39-40°C. It was very important that these limits were not exceeded, as a higher temperature could cause mortality amongst the snails while a lower one would not remove enough Chaetogaster. All the young snails to be treated were put into a nylon strainer and intermittently agitated vigorously in the heated water for 4-5 minutes. Between the brief periods of agitation the strainer was raised out

of the water and lowered into it again, several times, so that the parasites were washed out of the snails. In heavy infestations they were seen falling to the bottom of the beaker in large numbers. After this treatment, which removed a high percentage of the Chaetogaster, the snails were returned to another beaker, ready to be infected the next day. Less than 1% of the snails were killed by this heat treatment and none of the difficulties described by Khalil (1961) or Coyle (1961) were encountered; high levels of infection were achieved in every case as described below.

Meanwhile another method of controlling, if not eliminating, Chaetogaster was described by Berger (personal communication, 1967) who used the guppy (Lebistes reticulatus) for this purpose. The natural history and management of this small fish, which is common in Kenya, have been described by Fraser-Brunner (1954). Before these fish were put into the aquaria with the snails a series of experiments were carried out. In the first of these guppies were introduced into an aquarium with L. natalensis which were heavily infested with Chaetogaster. After a very short period they were seen to pick them off the snails, and the smaller ones were especially active in this respect. In another experiment 30 Chaetogaster, which had been removed from L. natalensis during heat treatment, were placed in a petri dish with one small

guppy, approximately 2 cm. long, which was seen to eat them all in 10 minutes. This experiment was repeated several times with the same result. Individual snails, heavily infested with Chaetogaster, were then placed in beakers with guppies which darted up to the snails to pick off the Chaetogaster before dashing away again. The fish appeared to be "attacking" the snails but they did not seem to be disturbed by this and continued feeding normally.

These experiments showed that guppies will eat Chaetogaster, and that they will also remove them from L. natalensis, apparently without disturbing the snails. According to Coe (personal communication, 1967) guppies do not eat young snails or egg masses, and experiments were carried out to see if newly hatched snails or metacercariae would be taken in certain circumstances.

Experiment 6.1: After immersion in boiling water, to kill any contaminant snails, aquatic plants were put into a clean beaker which was then filled with water which had previously been filtered through Whatman's No. 1 filter paper. Eleven guppies, males and females, large and small, were also put into the beaker together with 10 newly hatched L. natalensis and an egg mass which contained 10 embryos. When the experiment was terminated 3 days later the 10 newly hatched snails were recovered alive; the embryos in the egg mass had not hatched but the latter had been damaged, apparently in handling, and 4 embryos were missing.

This experiment was repeated on two occasions and the egg masses remained undamaged.

Experiment 6.2: Three egg masses containing undeveloped embryos attached to a Nymphaea sp. leaf, together with another containing 20 embryos in the process of hatching, were put into a 2 litre beaker with 14 guppies, males and females, large and small. The water had been filtered as described previously and the Nymphaea sp. leaf examined for young snails. Five days later the experiment was terminated when 12 of the young snails were still alive and one damaged shell was also found. The egg masses were undamaged.

Experiment 6.3: Seventeen guppies, males and females, and all sizes, were put into a small tank with filtered water and a Nymphaea sp. leaf with 2 egg masses attached to it. One of these contained 19 and the other 20 embryos. When the experiment was terminated 4 days later no hatching had occurred in one egg mass which was intact, but the 19 embryos in the other had all hatched and 18 were found alive.

Experiment 6.4: In this experiment 7 guppies, both sexes and of all sizes, were put into a 1 litre beaker with filtered water, and a piece of grass with 125 metacercariae attached to it was floated on the surface. Three days later the experiment was terminated and 122 metacercariae were found to be still attached.

In these experiments controls were set up which

were similar in every respect but without the fish. Normal development took place in these controls.

Conclusions:

1. Egg masses. These were damaged in 1 of the 3 experiments and 40% of the embryos lost. The damage was thought, by its nature, to be due to daily handling and not due to the guppies.

2. Newly hatched snails. In one experiment there was no reduction, but this was 40% and 5.3% respectively in the other two. It would therefore seem that under certain conditions newly hatched L. natalensis may be eaten by guppies.

3. Metacercariae of F. gigantica. In the single experiment there was a reduction of 2.5%. Some of this loss may have been due to trauma as the metacercariae were counted daily, however there was no loss in the control experiment which was handled in the same way. The guppies themselves could also have been responsible for dislodging the cysts from the floating grass. The sediment in the beaker was not examined for such metacercariae.

As Pellegrino, De Maria and De Moura (1966) had shown that guppies eat the cercariae of Schistosoma mansoni this work was not repeated with F. gigantica. It was assumed that they probably would be eaten, but that the chances of this being of importance would probably be slight as the cercariae very soon encyst. It was not known if the fact that most cercariae of

F. gigantica are shed during darkness would have any protective value.

The guppies thrived under laboratory conditions in the aquaria with the snails.

They were fed daily with 'Tetramin' (Tetrakraftwerke) which does not contain copper ions and is also suitable food for snails (Baensch, personal communication, 1967). These fish appeared to reduce the number of Chaetogaster but it was decided to examine this experimentally.

Experiment 6.5: Ten L. natalensis, heavily infested with Chaetogaster, were put into an aquarium with several guppies of both sexes and all ages. Ten others of similar size from the same aquarium were left without guppies, as controls. After only 3 days no Chaetogaster could be seen in the snails kept with the guppies, although the controls were still seen to be heavily infested. One snail from each group was killed every 3 days, broken up, and all Chaetogaster recorded. The 10 snails which had been kept with the guppies yielded only 12 Chaetogaster all of which seemed to be adults, the mean number thus being 1.2. The control snails contained a mean of 62 Chaetogaster each. It was therefore concluded that the guppies greatly reduced the number of Chaetogaster per snail, but that elimination might not be achieved from a population for some time.

The first guppies were obtained in April, 1967,

The infection of snails

Both F. gigantica and F. hepatica are found in Kenya, and reference has been made to their respective occurrence in Chapter 2. In a recent survey Ogambo-Ongoma (1969) found that only 0.32% of Fasciola infections of slaughtered cattle were due to F. hepatica.

Dinnik and Dinnik (1955) stated that apparently L. natalensis cannot serve as an intermediate host of F. hepatica in Kenya.

F. gigantica eggs were collected from naturally infected cattle at the Kenya Meat Commission abattoir at Athi River, from the experimental cattle or from naturally infected sheep at a local slaughter house. As many flukes as possible were removed from each infected liver into physiological saline kept at about 40°C in vacuum flasks. The bile was also collected, flukes and bile from each animal being kept in separate bottles which were numbered.

At the laboratory the flukes were incubated at 40°C for 2 hours to allow further egg laying. They were then examined and the eggs in those bottles containing F. hepatica were discarded. All the other collections were pooled and the flukes removed by sieving, while all unwanted material was removed by further sieving or sedimentation in tap water. The washed eggs were put into an incubator in the dark at 26°C in 500 ml. beakers which were $\frac{3}{4}$ filled with tap

water. Periodically the water was changed and the scum, which quickly developed on the surface of the water during the first few days of incubation, wiped off the beakers.

Some general observations were made on the development of F. gigantica eggs so that enough miracidia could be available to infect snails when required. It was found that at 26°C a few miracidia hatched at 15 days, and that they hatched en masse by 17 days. This confirms the report by Dinnik and Dinnik (1959).

The technique used to stimulate hatching was to remove the eggs from the dark container, pour off as much of the supernatant water as possible and then ¼ fill the beaker with cold tap water. The beaker was then placed next to a strong light source. This combination of a drop in temperature and stimulation by light "triggered off" the hatching mechanism. According to Coyle (1961) either of these stimuli would cause hatching. Pouring off the supernatant water at the start also removed any miracidia which may have hatched in the dark. Therefore all miracidia later seen were newly hatched. After hatching, the temperature of the water in the beaker was raised to about 26°C and the infection of the snails was carried out at this temperature. Miracidia less than 4 hours old were always used to infect snails, and they were usually less than 3 hours old. Only apparently

normal miracidia were used.

All the batches of miracidia studied were positively phototropic, swarming in masses on that side of the beaker nearest the light source. This agrees with the findings of Manipol (1936), Alicata (1938) and Guralp, Ozcan and Simms (1964), but not with those of Kendall and Parfitt (1953) and Taylor (1964). It is possible that these findings are related to the occurrence of different physiological races of flukes.

For the infection of snails a drop of water from the beaker containing miracidia was removed to a small petri dish with a fine-drawn pasteur pipette. This drop was examined with a hand lens, and the miracidia counted and adjusted to the required number. The drop was kept as small as possible to facilitate examination. In nearly every case snails were infected singly and were from 4-8 mm. long; occasionally snails as small as $2\frac{1}{2}$ mm. and as large as 11 mm. long were used to make up the required numbers. The infective dose was usually one miracidium per 1 mm. length of snail, with a maximum of 8 miracidia. However, in the first infection of all (3-4-67), approximately twice this dose was employed.

Standen (1963) found that L. natalensis are susceptible to infection with F. gigantica at all ages but that half-grown specimens are the most suitable. He exposed snails individually or en masse to 5-10 miracidia per snail. Dinnik and Dinnik (1959)

however stated that young L. natalensis can be infected much more easily than older specimens; they exposed individual snails to 5 miracidia each.

A snail of suitable size was placed in the drop of water with the miracidia and left for a few minutes before the petri dish was half-filled with water. The snails were left with the miracidia for about 3 hours, so that infection could take place, and were then removed to an aquarium. After about an hour in the petri dish there was a tendency for snails to crawl out, so that the petri dishes had to be placed in a tray which also had water in it.

As it was not practicable to collect F. gigantica eggs for each infection various ways were devised to delay hatching until the miracidia were required. Dinnik and Dinnik (1959) reported that the embryo was much less susceptible to the effect of low temperatures after it had developed past the earliest stages, and so all the egg cultures were initially kept at laboratory temperature (16-23°C) or in the incubator (26°C). Thereafter they were kept in the dark at lower temperatures. In this way hatching could be delayed up to 103 days, the longest required in this work. It was very important not to stimulate hatching unintentionally by exposure to light when the water in the cultures was changed; however at lower temperatures it had to be changed very infrequently. It was found that the eggs of F. gigantica, kept at

temperatures below about 21°C, did not hatch to any extent in the dark over these periods. There was no obvious loss of ability to infect snails when hatching had been delayed for these long periods, and this confirms the report of Dinnik and Dinnik (1959).

Fungal infections in F. gigantica eggs

At the start of this work it was seen that incubating eggs of F. gigantica often appeared to be invaded by fungi. Mycelial tubes and empty sporangia were seen within the eggs. The following observations were made on this phenomenon. The fungus appeared to invade the egg early in the incubation period, possibly even before it left the liver fluke, and could be seen clearly after a few days of incubation at 26°C. There was no evidence that fungal invasion ever occurred after the miracidium had become recognisable as such and, in particular, dead or living miracidia were never seen entangled in mycelia. Buckley and Clapham (1929) studied the invasion of helminth eggs, including F. hepatica, by fungi. The fungi which infected the ova of F. gigantica were never positively identified although they closely resembled the illustrations of Catenaria anguillae in the article. These authors further stated that the source of infection was tap water, and also showed that a culture of uninfected F. hepatica eggs in sterile distilled water, became progressively infected on incubation with 5 sporangia of Catenaria anguillae in Diphyllobothrium

eggs.

Both Coyle (1961) and Taylor (1961) referred to the invasion of F. gigantica eggs by fungus but gave no details. Dinnik (personal communication, 1967) considered that infection occurred within the fluke itself.

On occasions over 99% of the cultured eggs were infected with fungus but in no case in this work was the infection of snails prevented or curtailed. It was always possible to obtain enough miracidia for this purpose.

Experiment 6.6: Eggs were collected from the gall bladders, bile ducts and flukes from 5 cattle. They were kept separate at all times, and after washing with water from the same tap, were incubated at 26°C for 11 days. On examination it was found that the range of fungal infections was approximately 8% to 30%. These results indicate that the infection rate varies in different animals, under these conditions.

Experiment 6.7: Eggs were collected and washed as described in the previous experiment. However the collections were not made on the same day. The results are shown in Table 6.1 and provide evidence that the infection rate is related to the temperature of incubation.

F. gigantica eggs of ovine origin seemed to have at least as high a rate of infection as those of bovine origin.

Table 6.1

Infection rates with fungus in F. gigantica eggs kept at different temperatures

Animal	Incubation Temperature (°C)	Time (days)	Infection rate(%) (Approximate)
A805	16-20	73	24
B449	20-26	40	99
B458	20-26	42	99
6 animals (pooled)	26	14	99

It was not possible to determine whether this infection arose from the tap water, by infection in the liver or in the fluke itself because lack of time precluded further studies. However, these observations do show that infection does not necessarily occur in the alimentary canal of the host.

Infection rates and mortality of infected snails

At the start of this work observations were made on the longevity of infected snails, and on the infection rates, so that some idea could be obtained of the numbers that had to be infected to produce the required numbers of metacercariae. Khalil (1961) and Coyle (1961) considered that Ch. limnaei prevented infection so it was essential to know if the Chaetogaster problem had been overcome.

If they are to shed cercariae snails must not only

be infected but must also live long enough after infection. Dinnik and Dinnik (1956) had shown that at 26°C the emission of cercariae starts at 36-40 days after infection with 3-5 miracidia and this observation was confirmed, the earliest shedding being noticed 37 days after infection, satisfactory sheddings occurring after 44 days. Plate 6.3 shows an infected and an uninfected L. natalensis.

In this work a total of 4413 snails were exposed to infection, 3544 to miracidia of bovine origin and 869 to miracidia of ovine origin. Forty three separate batches of snails were infected, a mean number of nearly 103 snails per batch. The snails were selected for infection by size only as it was not practicable to do this on an age basis.

The mortality rates of snails exposed to infection varied but in only one case did nearly all the snails die before the date when cercariae were expected to be shed. This was the first batch, which was exposed to infection with 2 miracidia per mm. length of snail and only 5 snails were alive after 9 days. It was thought that the infective dose was too heavy and this was reduced to 1 miracidium per 1 mm. in all subsequent infections, but other factors may have been involved for death to occur so early in the infection. A period when unusually high mortality occurred has already been referred to in connection with mortality in the guppies. This was thought to have been due to

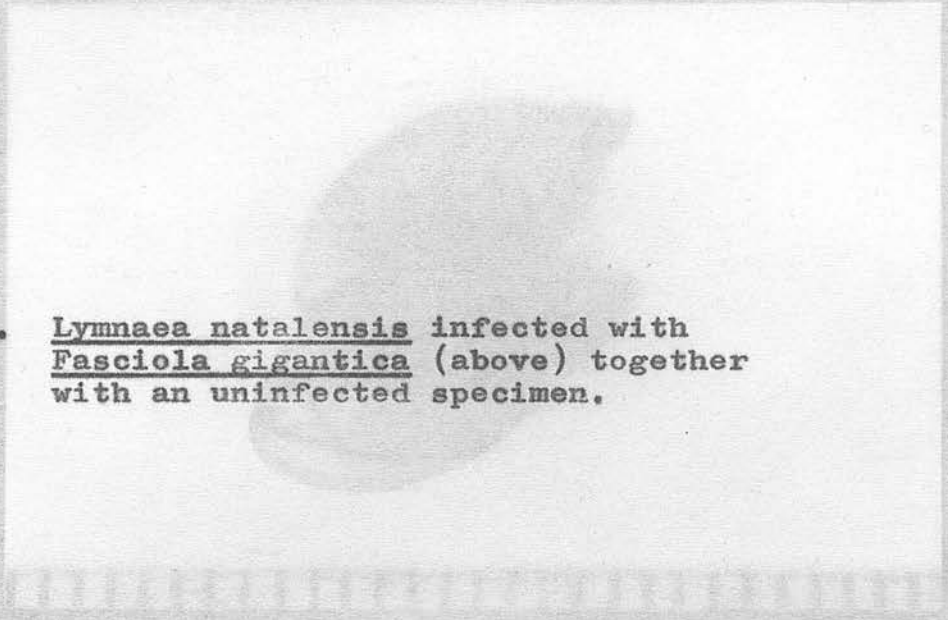


Plate 6.3. Lymnaea natalensis infected with Fasciola gigantica (above) together with an uninfected specimen.

contaminated water and mortality rates returned to normal after about a month.

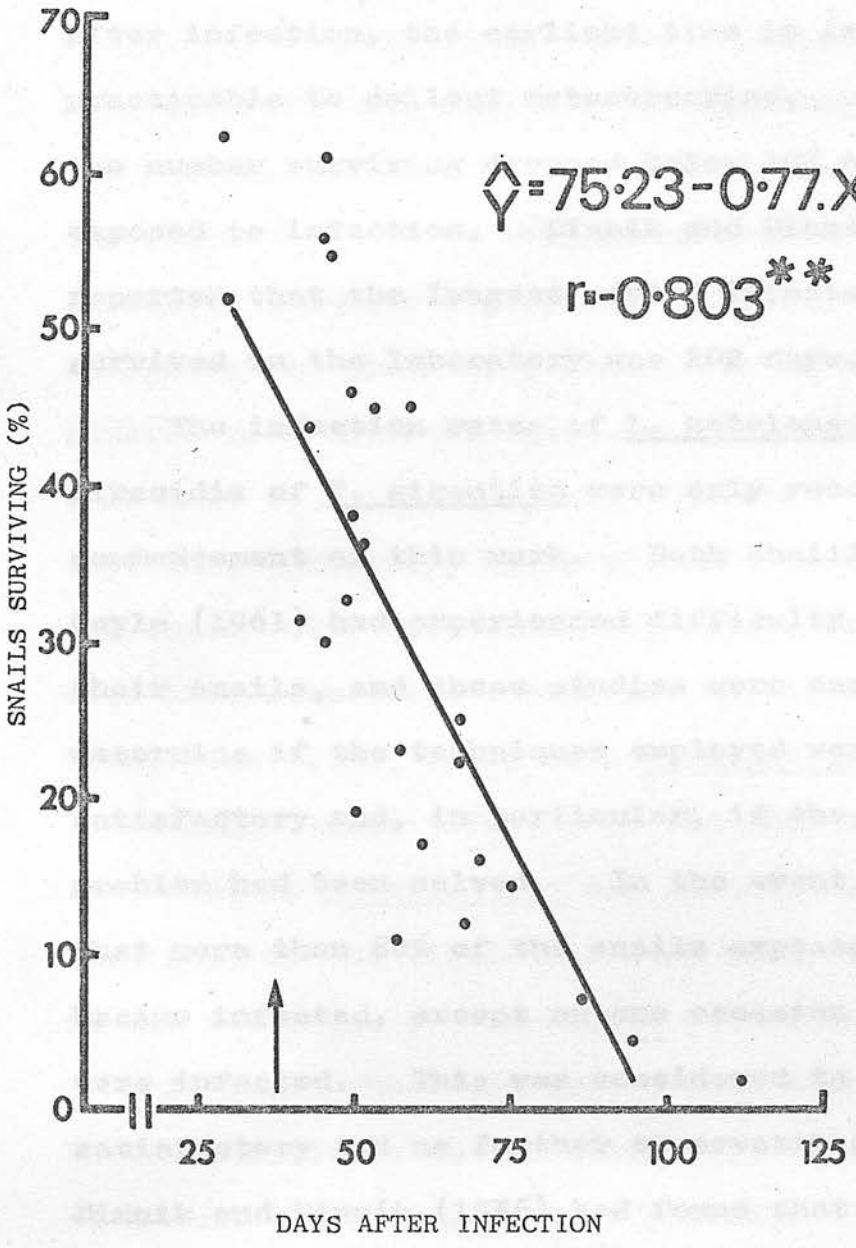
The longevity of L. natalensis after exposure to infection with miracidia of F. gigantica, and the mean survival times, are shown in Table 6.2.

Table 6.2

Longevity of Lymnaea natalensis after exposure to infection with miracidia of Fasciola gigantica

Batch Number	No. of Snails	Days post-infection	No. and percentage of snails alive	
7-4	77	43	34	44.1
		50	28	36.3
17-4	38	46	21	55.2
		52	17	44.7
10-5	121	31	62	51.2
		47	40	33.0
		55	28	23.1
20-5	153	45	86	56.2
10-5, 20-5	274	66-76	44	16.0
		81-91	18	6.5
		91-101	10	3.6
		107-117	2	0.7
10-7	136	46	83	61.0
		57	62	45.5
		67	31	22.0
11-7	138	49	52	37.6
		62	24	17.3
		69	17	12.3
4-8	98	40	31	32.0
		50	19	19.3
		56	11	11.2
13-8	121	31	75	62.0
		48	56	46.2
		67	31	25.2
		75	17	14.0
14-9	171	44	51	29.8

Graph 6.1 illustrates the mean survival times and shows approximately 40% of the snails to be alive 45 days



GRAPH 6.1 MORTALITY IN INFECTED SNAILS

after infection, the earliest time it is usually practicable to collect metacercariae. After 80 days the number surviving dropped below 10% of those exposed to infection. Dinnik and Dinnik (1956) reported that the longest period infected snails survived in the laboratory was 202 days.

The infection rates of L. natalensis exposed to miracidia of F. gigantica were only recorded at the commencement of this work. Both Khalil (1961) and Coyle (1961) had experienced difficulty in infecting their snails, and these studies were carried out to determine if the techniques employed were satisfactory and, in particular, if the Chaetogaster problem had been solved. In the event it was found that more than 80% of the snails exposed to infection became infected, except on one occasion when only 46% were infected. This was considered to be satisfactory and no further observations were made. Dinnik and Dinnik (1956) had found that 84% of L. natalensis exposed to 3-5 miracidia each became infected. However they did not refer to Ch. limnaei in their work.

Collection and administration of metacercariae

Standen (1963) reported that the L. natalensis-F. gigantica association does not respond as well as the L. truncatula-F. hepatica one to the reduced temperature stimulation technique for cercarial emergence. He found that the cercariae were

frequently discharged at night. Manipol (1936) had also observed that darkness irrespective of the time of day favours the shedding of cercariae. In the experiment to find if very young metacercariae are infective (Chapter 4), it was observed that cercarial emergence was pronounced soon after the snails were removed from daylight into the dark incubator. None were seen to be emitted while the snails were in daylight. However, it is possible in this case that the fresh water into which the snails were put might also have stimulated emergence, as Kendall and McCullough (1951) found with F. hepatica. On the other hand Taylor (1961) reported that the shedding of cercariae of F. gigantica was induced by direct sunlight, or the light from a 60 watt electric bulb. He did not refer to the effect of darkness however.

It would therefore appear that darkness is but one of the stimulants to the emergence of cercariae, rather than that there is a form of periodicity or biological rhythm involved. It is also possible that different physiological races of flukes may require different stimulants for the emergence of cercariae.

As already described, about one week before the collection of metacercariae started, guppies were put into the aquarium to reduce the number of Chaetogaster present. For shedding, the snails were collected into clean beakers about ¾ filled with fresh water and several pieces of freshly cut grass were floated on the

surface. Suitable local grasses were Common Star Grass (Cynoden dactylon, Pers) or Kikuyu Grass (Pennisetum clandestinum, Hochst). It was very easy to count the cysts on these grasses which have a rib along the centre, moreover these grasses are available at È.A.V.R.O. at all seasons. Urquhart (1954), Standen (1963) and Bitakaramire (1968), however, used plastic strips or sheets for collection.

The cysts of F. gigantica are grey-white when fresh, becoming grey-brown with age, and the contrast of these cysts against the dark-green grass made counting very easy with a hand lens. However, a stereoscopic microscope had to be used if the grass had become yellow-brown with age.

The snails were left in the beakers overnight at 26°C and returned to the aquaria in the morning. Nearly all the metacercariae encysted on the grass under these conditions, very few encysting on the sides of the beakers. The pieces of grass were routinely left in their respective beakers for one day to allow time for the cysts to become firmly attached to the grass. They were then removed, lightly brushed to dislodge debris and snail faeces, etc. and transferred to clean beakers with fresh tap water. They were kept at the ambient temperature in the shade, and care was taken to be sure that the metacercariae were kept under the water.

All uninfected snails which had been exposed to

infection were destroyed, so that any chance of selecting strains which were genetically resistant to infection would be avoided.

When metacercariae were required for the infection of experimental animals some were examined under the microscope for viability. It is impossible to tell if a metacercaria is both viable and capable of infecting the final host; even if it is shown that it can encyst in vitro it is not necessarily vigorous enough to infect. The criteria adopted were that those metacercariae which exhibited the paired cystogenous glands with sharply defined margins were assumed to be capable of infecting the final host if administered before they were 2 weeks old. At this time, of the 300 which were so examined before each of the experiments, less than 0.25% showed any deterioration. However, Coyle (1961) found that a high proportion of non-viable metacercariae occur.

The infective doses were then counted using a hand lens, care being taken not to overheat the metacercariae. Any doubtful cysts or free metacercariae were removed.

The use of a possible bovine-adapted strain with sheep, or vice versa was avoided except in calf C37 (Chapter 14).

For infection of both cattle and sheep, the pieces of grass were shaken free of surplus water and rolled up in freshly made dough (wheat or maize flour

mixed with water). The infective bolus was placed at the back of the animals' tongue which had been pulled forward. The tongue was then released and the bolus swallowed. In every case an examination was made after a few minutes to make sure that the infective dose had all been swallowed.

The techniques used were very effective when judged by the high recovery rate of flukes as a percentage of the metacercariae which were administered.

CHAPTER 7

Laboratory Materials and Methods

1. Measurements of weight gain of experimental animals

The animals were weighed at weekly intervals in the experiments at E.A.V.R.O. but only at irregular intervals at Edinburgh. All weighings were carried out from 0800-1000 hours.

2. Examination of faeces for evidence of parasitism

The method used was the direct differential centrifugal flotation (D.D.C.F.) technique. About 5 grams of faeces were broken up and washed through a sieve with about 20 ml. of water. This fluid was then centrifuged at about 1000 r.p.m. for one minute, the supernatant discarded and replaced with saturated salt solution. The sediment was then suspended and recentrifuged. The centrifuge tube was carefully topped up with saturated salt solution until a slight convex meniscus was formed. A cover slip was placed over the meniscus, left to stand for about 15 minutes, and then lifted off vertically and placed on a microscope slide for examination.

This method is suitable for the detection of nematode eggs and larvae and cestode eggs only. For trematode eggs the saturated salt solution was then poured away, the sediment resuspended in zinc sulphate solution, of specific gravity 1.3, and recentrifuged.

It was then topped up with this solution until a slight meniscus was formed, and a cover slip was placed over this. The tube was left to stand for 10 minutes before the cover slip was removed and examined as previously.

In Edinburgh an alternative method was developed when the only aim was to find Fasciola eggs. In this technique about 5 grams of faeces were well broken up and then differentially sieved, first through a 60 mesh, then through a 100 mesh and on to a 300 mesh sieve. The sieves used were Test Sieves to BS 410 (Endecotts or Greening). The washed faeces were examined in a petri dish which had previously been marked on the underside with parallel lines. A stereomicroscope was found most suitable for this. Alternatively, the washed faeces were dispensed into test tubes and centrifuged successively in water and zinc sulphate solution (specific gravity 1.3), and any Fasciola eggs were recovered from the surface as previously described.

At E.A.V.R.O. the method used for the examination of faeces for Fasciola eggs was that of Dinnik and Dinnik (1963). This is essentially a sedimentation technique.

3. Faecal egg counts

Counts of nematode eggs were carried out on the faeces of both cattle and sheep by a modified McMaster

technique (Dunn, 1969) in which 3 grams of faeces were weighed out in a bottle which was then filled up to the 45 ml. mark with saturated salt solution. To remove some of the faecal colouring matter, and so give a clearer picture in the counting chamber, the weighed faeces were first centrifuged in water which was then discarded.

Techniques for counting trematode eggs in faeces have been developed by many workers (Willmott and Pester, 1952; Dennis, Stone and Swanson, 1954; Dorsman, 1956; Coyle, 1958; Boray and Pearson, 1960; Dinnik and Dinnik, 1963 and Bitakaramire, 1967, are some of these).

Coyle (1958, 1961) attempted to overcome the problem of the low egg counts which he found to be usual with Fasciola gigantica in cattle, by using a special apparatus which enabled running water to be used for a sedimentation technique. Dorsman (1956) also developed a technique which involved a special apparatus for counting F. hepatica eggs in cattle faeces.

However it was considered that the technique chosen should be as simple as possible and should preferably not require any special apparatus. For this reason, the selective sieving method of Willmott and Pester (1952) was modified for use at E.A.V.R.O. It was found satisfactory for both cattle and sheep faeces. This method will now be described

as used with cattle faeces.

All faecal samples for Fasciola egg counting were collected between 0830 and 0900 hours. This was because Dorsman (1956) found that the number of eggs of F. hepatica in the faeces of cattle rises during the morning hours, and Guralp (1969) also showed a considerable diurnal variation in the number of eggs of F. gigantica in the faeces of a calf and a sheep. Approximately 200 grams of faeces were collected from each animal and well mixed, to ensure an even distribution of the eggs, before a 5 gram sample was weighed out. This was then well washed with tap water through a sieve of 250 microns (μ) aperture (BS 410: 1962, No. 60, Greening (Hayes)), on to a sieve of 53 μ aperture (BS 410, No. 300, Endecotts (Test Sieves) Ltd.). The faecal debris, eggs and silt were then washed out into a beaker. The egg counting was carried out in marked petri dishes as previously described, a drop of 1% methylene blue being added to make the liver fluke eggs show more clearly. In a further development the washed faecal debris was made up to 100 ml., well mixed, and a 20 ml. sample of this was taken for counting - this containing the eggs from 1 gram of faeces. As the number of eggs per gram of faeces was so much higher in sheep, the equivalent of only 0.1 gram of faeces was used for the egg counting.

The method later described by Bitakaramire (1967) was essentially the same except that he used sieves

with apertures of 211 μ and 64 μ. Dinnik (1958) found that the eggs of F. gigantica in Kenya were 150 to 190 μ long and 79 to 100 μ wide and for this reason it was decided to use sieves with apertures of 250 μ and 53 μ so as to be certain that all eggs passed through the larger sieve and that all were retained by the lower one. Bitakaramire (1967) reported a recovery rate of from 70% to 93% with his technique. Although no critical testing was carried out with the described technique it gave very reproducible results in practice, and was easy and simple to use although rather time consuming. When the sieving method was being used to examine faeces for evidence of infection, the sieves were dried in the hot air oven at 160°C, between examinations, so that any eggs which had been retained were destroyed.

Another method was also used for counting Fasciola eggs in faeces at E.A.V.R.O. This had been developed by Dinnik and Dinnik (1963) and also gave very reproducible results. It required considerable skill, so that its use had to be restricted to an experienced technician.

In Edinburgh the method adopted was that already in use in the laboratory, and was essentially a quantitative D.D.C.F. technique. In this, 5 grams of sheep faeces were macerated in a plastic bottle which was then filled to a mark at 125 ml. with water. After mixing well, 25 ml. were quickly removed, washed through

a coarse strainer, poured into a measuring cylinder and made up to 100 ml. with water. After shaking well, 12.5 ml. were measured into a test-tube in which the faeces were centrifuged successively in water, saturated salt solution and zinc sulphate solution (specific gravity 1.3). After the final centrifugation the meniscus was made up with further zinc sulphate solution and a cover slip was placed over it. The tube was left to stand for 10 minutes, after which the cover slip was removed vertically and the eggs washed off with distilled water into a ruled counting chamber.

While this method gave very clear preparations it also had several defects. One of these was that an irregular proportion of the fluke eggs adhered to the cover slip. After this had been discovered, a second cover slip was placed over the meniscus as soon as the first cover slip had been removed. This resulted in the recovery of up to twice as many eggs as had adhered to the first cover slip. A further smaller number could be recovered if a third cover slip was used, but this was too time-consuming and only two cover slips were used as a routine. The full investigation into these and other errors is given in Chapter 10.

At E.A.V.R.O. it was very important to be able to distinguish between Fasciola and paramphistome eggs. Their differences have been described by Dinnik (1958),

and Dinnik and Dinnik (1963).

4. Gall bladder egg counts

A dilution technique was used to estimate the number of eggs of F. gigantica in the gall bladders of the experimental cattle.

5. The extraction of flukes from infected livers

A. Mature infections

The common bile duct was ligated as soon as possible after death, and the small intestine - anterior and posterior to the point of entry of the duct - was examined for the flukes which were sometimes found there. Where the examination could not take place at once, the liver was stored entire at -15°C after the gall bladder had been opened and washed out. At the examination all visible bile ducts were cut open and the flukes removed (into 0.85% saline if they were alive). The liver was then cut into 1 cm. thick slices and squeezed, while fibrous areas were opened up to extract any further flukes. It was then cut into small cubes, which were squeezed again by hand before the debris was washed through a 10 mesh into a 30 mesh sieve, and the retained debris examined in aliquot#s in white trays so that any further flukes could be removed. In all cases with mature infections the whole liver was examined from both cattle and sheep.

B.1 Immature infections in sheep

The liver was cut into cubes about $1\frac{1}{2}$ cm. square

and a random sample was taken, which was weighed so as to form a known fraction of the weight of the liver (usually about a tenth). The selected cubes were squeezed in warm physiological saline and left, with periodic further squeezing, for about 4 hours at 37°C to allow the young flukes to migrate out. The liver sample was then sieved, using first a 10, then a 30 and finally a 60 mesh sieve. The residual matrix was put into fresh saline and left at 37°C overnight and again sieved. On each occasion the flukes were washed from the sieves into a beaker and aliquots were counted in a petri dish. As some flukes were broken, only the anterior ends were counted and the number obtained was multiplied by the appropriate factor to give the total estimated number of flukes. As it was found that some flukes started to distintegrate, after being left overnight in saline at 37°C, later liver samples were broken up and the flukes recovered and counted as soon after slaughter as possible. With sheep No. 11 the former technique yielded 87 and 81 flukes respectively in two samples, and 89 were recovered from another sample by the latter technique. The three samples were of equal weight.

Where both adult and immature flukes were present in the same liver, those in the bile ducts were removed as previously described, before the 1/10th sample was taken and examined.

B.2 Immature infections in cattle

The entire liver was always examined. In order to study the migratory pattern of the young flukes, selected livers were divided into three parts, as described by Ross, Todd and Dow (1966), and each part was treated separately. These were cut into 2 cm. slices and squeezed in physiological saline at 37°C, where they were left for four hours before being sieved, the fibrous matrix removed and the flukes recovered as previously described. The fibrous matrix of the liver was fixed in 10% formol-saline and examined again. It was not possible to use this technique in some of the experiments at E.A.V.R.O. as it was too time-consuming when several animals had to be killed daily. Therefore in these experiments all the livers were first weighed, the gall bladders were then incised and the bile measured and retained for egg counting, together with the gall bladder washings. The liver, gall bladder and hepatic lymph glands from each animal were weighed separately, and stored at -15°C until further examination could take place. For the examination, an amount of liver sufficient for one day's work was thawed out and broken up in water. It was then sieved and aliquots were examined in petri dishes. A final examination was carried out after the macerated liver had been put through a domestic meat mincer, using the finest screen.

In the experiment described in Chapter 13 four

cattle had both mature and immature infections. These livers were first examined for the former and then for the latter age groups, by the techniques already described.

6. Counting and measuring flukes

In Edinburgh, all flukes or fluke fragments with the oral sucker were counted, but it was found that a small proportion had lost this sucker, which was very rarely able to be recovered. Therefore at E.A.V.R.O. only those flukes which had the ventral sucker were counted (Roberts, 1968).

All flukes were measured unfixed and after death i.e. when they had lost all ability to contract. Under the conditions of the experiments it was impossible to measure the flukes in warm isotonic saline, immediately after removal from the body, as advised by Kendall (1965). A pilot experiment indicated that flukes did not show any evidence of contraction after they were recovered from livers which had been in the deep freeze for several weeks. Those flukes which were recovered from fresh livers were kept at 5°C until after death when they were measured. Measurements were carried out by laying the flukes on a smooth glass surface and measuring the length in millimeters with a clear plastic ruler. The accuracy of this method was confirmed using calipers. It was most important that sufficient moisture was present on the glass surface and that the flukes were

never stretched.

7. Mean dry weight of flukes

All the recovered undamaged flukes from one animal were macerated in distilled water in an Atomix Blender (M.S.E.). A known percentage of the volume of the evenly dispersed suspension was then dried to constant weight.

8. Post-mortem procedures

Post-mortem examinations were carried out on all animals as soon as possible after they had been killed. Only two animals died of fascioliasis. A careful examination was made of all internal organs for abnormalities, with particular attention to their colour and appearance. The lungs were examined for ectopic flukes and, if these were present, selected areas were removed for histological examination. The same procedure was followed for lesions found in other organs, except the liver which was removed and dealt with separately. The lungs were examined for Dictyocaulus viviparus which were retained for further examination. The rumen and reticulum were examined for paramphistomes, and the musculature of all cattle for Cysticercus bovis. In all cases where there was abscess formation, or other evidence of bacterial infection, specimens were submitted for examination.

In the experiment described in Chapter 10 the abomasum and the small and large intestines were

examined separately for helminths. The contents of each were washed through sieves of 10 and 60 mesh. The mucosa was rubbed under water to ensure that all parasites were removed. A tenth part of the sievings from the 10 mesh sieve were examined in a black tray, and a hundredth part of those from the 60 mesh sieve were examined under a stereoscopic microscope. All parasites were retained for identification. The same procedure was followed in the experiment described in Chapter 12. Where tapeworms were present representative specimens were stained and examined. The genera of the intestinal nematodes in the cattle were identified after clearing in lacto-phenol. In each case 100 randomly picked nematodes were identified from each part of the intestine.

In the experiment described in Chapter 10 the sternum, one femur, one ileum, and four to six lumbar vertebrae were bisected, and then photographed so as to display the extent of the erythropoietic tissue in the marrow cavities. Colour transparencies of the femurs were used to draw enlarged diagrams on cardboard, first of the outlines of the bone and then of the erythropoietic tissue. An estimate of the amount of active marrow in these femurs could then be obtained by cutting out and weighing first the whole bone and then the erythropoietic tissue, so that the relative areas and ratios could be assessed.

Samples of liver and other tissues were fixed in 10% formol-saline. From these samples sections stained with haematoxylin and eosin were prepared in the Department of Pathology, Royal (Dick) School of Veterinary Studies. At E.A.V.R.O. Von Kossa's technique (Culling, 1963) was used to confirm the presence of early calcium deposits in the bile ducts.

9. Photography

All the photography in Edinburgh was done by the Photography Department, Royal (Dick) School of Veterinary Studies, using Kodachrome IIA film for colour transparencies and Ilford R20 plates for black and white photographs. In Kenya Kodachrome X film was used for colour transparencies and Kodak Pan X Plus for black and white photographs.

All the entire livers were photographed soon after removal from the carcase and, in many cases, close-up photographs were also obtained of individual lesions (using extension rings on the camera). Nearly all the bovine livers were then cut into three parts, as previously described, and a slice about 2 cm. thick removed from the middle of each of these parts. Each side of each slice was then photographed, so as to demonstrate the distribution of the tracks in immature infections and the fibrous areas and thickened bile ducts in mature infections.

10. Haematology

Blood for haematological examination was collected

in vials containing disodium or dipotassium ethylenediamine tetra-acetic acid (E.D.T.A.; Sequestrene) apart from a small number of early samples collected into heparin. To minimise any changes in the constituents of the blood due to diurnal variations (Penny, Wright and Stoker, 1964) all sampling was carried out between 0830 and 0930 hours. The blood was always taken from the jugular vein. Whole blood and diluted blood were mixed by hand for at least 2 minutes before sampling in Edinburgh, and at E.A.V.R.O. a cell-suspension mixer (Matburn) was used.

A. Packed cell volume

In the experiment described in Chapter 9 this was determined with a Griffin-Christ Junior I centrifuge, fitted with a microhaematocrit tube head, at approximately 6000 r.p.m. for 15 minutes. In all other experiments a Hawksley microhaematocrit centrifuge was used. The capillary tubes were sealed by heat and centrifuged for 15 minutes after which the percentage cell volume was estimated using the reader provided with the instrument.

B. Haemoglobin concentration

This was estimated using the alkaline-haematin method of Clegg and King (1942). The optical density was read in an E.E.L. Portable Colorimeter (Evans Electroselenium) using a yellow-green Ilford 625 filter and compared with a standard equivalent to the colour of a 1:100 dilution of blood containing 16.0 grams of

haemaglobin per 100 ml. by iron content (Gibson-Harrison Artificial Standard, British Drug Houses).

C. Red cell counts

In the experiment described in Chapter 10 a Coulter Blood Cell Counter, Model A, Medical (Coulter Electronics) was used. The settings used were aperture 100, current 6 and threshold 10. The blood was diluted 1:100,000 with 0.9% sodium chloride solution.

All other red cell counts in sheep and cattle were done by the visual method of Dacie and Lewis (1963), using an improved Neubauer Chamber. In the experiments described in Chapters 9 and 10 at least 500 cells were counted, while in all the experiments at E.A.V.R.O. at least 1000 were enumerated. Dacie and Lewis (1963) discussed the errors in counting blood cells. It was considered that the standards of accuracy obtained were adequate as all counts were part of a series and were not performed in isolation.

D. Total white cell counts

Total leucocyte counts were done by a modification of the visual method of Dacie and Lewis (1963), using an improved Neubauer Chamber, 0.1 ml. of blood being added to 4 ml. of diluent. At least 100 cells were counted in Edinburgh, and at least 200 at E.A.V.R.O. Also at E.A.V.R.O., if the sum of the number of cells in one chamber differed by more than 10% from that in the other chamber of the haemocytometer, the results

were discarded and another two chambers were filled.

E. Eosinophil counts

These were done by a modification of the method of Dacie and Lewis (1963) using an improved Neubauer Chamber, 0.1 ml. of blood being added to 0.8 ml. of diluent. Two of the Neubauer Chambers were always filled and all the eosinophils counted. This method was only used in Edinburgh; at E.A.V.R.O. the number of eosinophils was calculated from the total white cell count and the differential count on a blood film.

F. Differential white cell counts

In every case two blood films were made by the method of Dacie and Lewis (1963) on microscope slides which had been suitably cleaned. The films were first fixed with Leishman's stain (G. T. Gurr) for 2 minutes, after which 2 volumes of distilled water buffered to pH 6.8 were added and well mixed. The diluted stain was left on for 5 to 15 minutes before being washed off and the preparation differentiated with buffered distilled water for up to 2 minutes. The differential white cell counts were made using the four field battlement technique in which 50 leucocytes are differentiated at each of the four corners of the smear (Penny, 1967). Thus 200 cells were differentiated on every smear, using an X100 oil-immersion lens.

G. Price-Jones curves

A limited number of these were drawn in the acute

fascioliasis experiment in sheep. An area of each film, where the cells were neither distorted nor shrunken, was selected and the diameters of 100 red cells measured using a calibrated eye-piece micrometer (Dacie and Lewis, 1963; Holman and Dew, 1963).

The packed cell volume was determined and the blood films made within 2 hours of a blood sample being collected. The cell counts were completed within 7 hours, and the haemoglobin estimations within 30 hours. The blood samples were stored at 4-6°C overnight. These times are well within the limits recommended by Archer (1965).

11. Collection and storage of serum

The blood samples for biochemistry were collected in universal bottles and allowed to clot in the incubator at 37°C for 2 hours, then kept at 4-6°C for up to 1 hour for the clot to retract. After making sure that the clot had separated, the bottle was centrifuged, the serum was then poured off and re-centrifuged to remove any residual red cells. By this technique the serum was always free from visible haemolysis. It was stored at -25°C until required. Some samples were allowed to clot at room temperature and then treated as described. The number of times the serum was thawed and refrozen was kept to a minimum. This was especially important in view of the large number of tests which were carried out. To this

end aliquots were kept in different bottles. Also those tests which were most sensitive to degenerative changes were carried out first.

12. Sheep plasma folate levels

These determinations were carried out by Dr. A. Omer at the Royal Infirmary, Edinburgh. He used the method of Waters and Mollin (1961), except that plasma was used instead of serum, and L-ascorbic acid was added to the standard as it is a non-specific stimulant of the Lactobacillus casei.

13. Sheep serum vitamin B_{12} levels

These were also estimated by Dr. Omer using the method described by Spray (1955).

14. Radioactive isotope studies

The methods using sodium chromate (^{51}Cr) solution BP and di-isopropyl phosphorofluoridate - ^{32}P ($DF^{32}P$) were those described by Sewell, Hammond and Dinning (1968).

15. Bromsulphthalein (B.S.P.) dye excretion test

This test was only carried out on sheep. Normally functioning liver removes this dye from the plasma proteins and excretes it into the bile. However, if the liver function is impaired or biliary obstruction is present, the excretion is delayed according to Seligson and Marino (1958), whose method was used for the determination of the concentration of B.S.P. in plasma. The dose of 5 mg./kg. body-weight

was injected into the jugular vein by hypodermic needle, and blood samples were collected from the opposite jugular vein at $1\frac{1}{2}$, $2\frac{1}{2}$, 7 and 10 minutes after injection. Readings were taken with an E.E.L. Portable Colorimeter with an Ilford 625 filter. Using a standard graph, calibrated using known quantities of B.S.P. measured in the same colorimeter, the readings were converted to milligrammes of B.S.P. per 100 ml. of plasma.

16. Serum biochemistry

The time during which the serum was stored at -25°C before each test was carried out is given with the results.

A. Serum glutamic oxaloacetic transaminase

The activity was measured on fresh serum in the experiment described in Chapter 9 by the method of Yatzidis (1960). Optical densities were determined with an E.E.L. Portable Colorimeter using an Ilford 623 filter. A standard graph was produced from known concentrations of pyruvic acid. For the experiments with cattle the modified Reitman-Frankel (1957) method was used with the reagents supplied in the Dade test kit (Dade Reagents). Their 'Enza-trol' known enzyme test control was used as a positive check, for each batch of sera tested. The readings were made with a Beckman model B spectrophotometer (Beckman Instruments) at a wavelength of 505 mμ. against a distilled water blank as zero. The calibration curve was set up with

the calibration standard and the specific substrate provided with the test kit. The results were all converted into International Units.

B. Serum glutamic pyruvic transaminase

The activity was measured by the method of Yatzidis (1960) and the optical densities determined with an E.E.L. Portable Colorimeter using an Ilford 623 filter.

C. Alkaline phosphatase

The method of Kind and King (1954) was used and the results were expressed as King-Armstrong Units.

D. Sorbitol dehydrogenase

The activity was determined by the method described by King (1965), and the results expressed as International Units. It was a modification of the colorimetric assay of Sevela and Tovarek (1960, 1961).

E. Iodine liver function test

The technique described by Lloyd (1957) was used.

F. Total serum protein

This was estimated by the biuret method (Reinhold, 1953), standardised by the Kjeldahl method. The results were given as grams per 100 ml.

G. Serum bilirubin

This was determined by the method of King and Coxon (1950) and the results expressed as mg. per 100 ml.

H. Ornithine carbamyl transferase

The activity was determined by the microdiffusion

NH_3 method of Reichard and Reichard (1958) and the results expressed as International Units. The standard solutions used to prepare the graphs were also put through the Conway Units. The values were read with a Beckman model B spectrophotometer at a wavelength of 480 mu.

I. Electrophoresis

This was performed on cellulose acetate strips (Millipore Filter Corp.) 1.5 cm. x 17 cm. in a Shandon Electrophoresis Apparatus (Shandon Scientific Co.), Barbital buffer of pH 8.6 was used, and a constant current of 0.4 milliamps per cm. width of cellulose acetate strips was passed for $2\frac{1}{2}$ hours. The serum was applied to each strip with a Spinco applicator (Spinco Division, Beckman Instruments) and after some preliminary runs 5 µl. of a 1:4 dilution was found to be suitable. The strips were stained in a bath of lissamine green (Brackenridge, 1960) and then differentiated in 5% acetic acid. After air drying, they were scanned and integrated using a Chromoscan (Joyce, Loebl).

J. Serum glycoproteins (protein bound hexose)

The technique used was that of Weimer and Moshin (1953). The results were expressed as mg. per 100 ml.

CHAPTER 8

Experimental Animals

Feeding and husbandry

A. Sheep

(1) Acute Fasciola hepatica infection: Adult Blackface wethers from a farm with no history of fascioliasis, were brought indoors and dosed on two occasions with 100 mg. of thiabendazole ('Thibenzole', Merck Sharp and Dohme) per kg. live-weight. They were thereafter kept on a litter of wood shavings, and the concrete-floored pens were cleaned weekly so as to minimise re-infection with parasitic nematodes. The sheep were fed on a daily ration of hay and water ad lib., plus 1½ lb. per head of a 4:1 mixture of barley and Cattle and Sheep Protein Concentrate Pellets (Scottish Agricultural Industries).

(2) Chronic F. hepatica infection: Nine Cheviot wethers were treated as described by Sewell, Hammond and Dinning (1968). During a period of respiratory infections, to be referred to later, the pens were cleaned out daily in the hot summer weather to minimise the amount of ammonia given off.

(3) Chronic Fasciola gigantica infection: Twenty-one 9-month-old Merino x Corriedale wethers were obtained from a farm which had been free from known fascioliasis for some years. They were housed at night because of the danger from wild carnivores, but

grazed all day. Water was supplied ad lib. at all times, but hay was only given in the houses at night (ad lib.) and on the pastures when the grazing was insufficient. No concentrates were fed, but 'Maclik' mineral bricks (Cooper, McDougall and Robertson (East Africa)) were available in the house at all times. Three samples of the hay were found to contain 6.83, 4.90 and 4.76% of crude protein calculated on a dry matter basis. These analyses were carried out by the Animal Production Division, E.A.A.F.R.O.

The pastures were predominantly a dense Star Grass (Cynoden dactylon) sward with patches of Kikuyu Grass (Pennisetum clandestinum). Other grasses were also present in lesser amount - Setaria sphacelata, Themeda triandra and Digitaria scalarum. There was one large patch (about $\frac{1}{4}$ acre) of Solanum incanum, and occasional Lippia sp., Leonotis sp. and other herbs, as well as occasional patches of sedges (Cyperus sp.). There were some short-grazed nutritious areas and large tall rank little-grazed areas of pasture. Mr. A. McKay, who examined the grazing areas, reported that they were good permanent grass pastures with marked seasonal variation in productivity and nutritional value. They were rather undergrazed so that the tall rank areas had provided scope for selective grazing. Although there were no legumes there were some browsable herbs which might increase the crude protein content of the diet, especially in drier periods. He

considered that it was likely that only maintenance could be expected during the driest part of the year, but there was green grass for long periods and cattle (see below) should be capable of liveweight gains of up to 1 or 1½ lb. a day.

B. Cattle

Chronic F. gigantica infections: Nearly all the calves were Guernsey-type castrates and had arrived at E.A.V.R.O. when they were 3 to 4 months old. They had been reared on a farm under conditions in which infection with Fasciola was highly unlikely. They were housed at night. When 6 to 7 months old they were left out at night, but after only 3 weeks one was killed by a wild carnivore and so this practice was discontinued. Water was available at all times and lucern hay was given ad lib. at night. A mineral supplement ('Maclik for Cattle and Sheep', Cooper, McDougall and Robertson (East Africa)) was mixed with the concentrate ration as recommended by the manufacturers, and was also available in brick form. The concentrate ration fed was Young Stock Pencils (Unga), which contained 16.4% protein, and a vitamin/mineral supplement 'Vitamealo'. It was fed to each calf in the experiment described in Chapter 12 at 1½ lb. per day, increasing to 5½ lb. by the time they were 7 months old and continued at this rate until slaughter. The same initial routine was followed in the experiment described in Chapter 13, but after they

were 9 months old the concentrate ration was progressively reduced, ceasing when these calves were 10½ months old.

The calves were at pasture all day. The grazing was that which was later used for the sheep and which has already been described. A few calves, also Guernsey-type castrates from the same farm, were brought in at 10 to 12 months of age. As these were found to be infected with Anaplasma and/or Babesia, they were kept separate from the other calves. It was impossible to house them at night, and no supplementary feeding was given. The composition of the pasture was very similar to that described previously except that it was even more undergrazed and had less Cynoden but more Setaria and Themeda.

Meteorological observations

These were obtained from the Physics Division, E.A.A.F.R.O., and are given in Tables 8.1 and 8.2. Both E.A.A.F.R.O. and E.A.V.R.O., where the experimental animals were kept, are at Muguga which is 1°13' South, 36°38' East, at an altitude of 6875 ft.

A. Rainfall (mm.)

Table 8.1

Rainfall at Muguga

(to nearest mm.)

See overleaf/...

	1967	1968	1969
January	Nil	Nil	66
February	4	109	49
March	22	238	79
April	419	240	60
May	376	219	159
June	31	54	10
July	13	2	16
August	35	1	17
September	34	9	
October	81	32	
November	97	212	18 years
December	50	130	average 1013
Total	1161	1243	

B. Air temperature (°C)

Table 8.2

Air temperatures at Muguga

(to nearest °C)

	1967 Monthly means of daily values			1968 Monthly means of daily values			1969 Monthly means of daily values		
	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean
January	24	11	17	24	9	17	22	10	16
February	25	11	18	23	11	17	21	11	16
March	25	12	19	21	11	16	21	12	17
April	22	12	17	20	11	16	23	12	17
May	20	12	16	19	11	15	21	11	16
June	18	9	14	18	9	14	20	10	15
July	18	9	13	17	8	12	19	10	14
August	18	8	13	17	8	13	19	9	14
September	20	9	15	21	8	14			
October	21	11	16	22	10	16			
November	20	11	16	19	11	15			
December	22	10	16	21	10	16			
Mean	21	10	16	20	10	15			

Reasons for concluding that Fasciola was not transmitted at E.A.V.R.O.

Dr. J. A. Dinnik, who had been at E.A.V.R.O. since it was moved to Muguga in the early 1950's, stated (personal communication, 1967) that transmission of Fasciola had never been known on the estate and further that no Lymnaea sp. had ever been found there outside the laboratory. All water is pumped from bore-holes, and piped to troughs fitted with ball-valves. These troughs were examined for snails periodically but none were ever found. There is no surface water, and only very temporary wet muddy areas occur, confined to the times of heaviest rain-fall. These are in any case unsuitable habitats even for Lymnaea truncatula, which Dinnik and Dinnik (1958) had shown can serve as a snail host of F. gigantica as well as F. hepatica.

In the experiments to be described there was no indication that natural intercurrent infection had occurred, as the prepatent periods were all within a narrow range and uninfected control animals showed no evidence of infection at slaughter. Finally, at the close of all experimental work, two animals, A354 (4 years old) and 2776 (12½ years old), which had been born on the estate and had never left it, were slaughtered and no evidence of Fasciola infection was found in them.

Prevention of extraneous disease

A. Edinburgh

All sheep were immunized with 'Agnulin' (Glaxo Laboratories).

B. E.A.V.R.O.

(1) Sheep: They were immunized with Rift Valley Fever and Bluetongue vaccines from the Kenya Veterinary Laboratories, Kabete; 'Blanthrax' (Anthrax and Black-quarter vaccine) and 'Covexin' (Burroughs Wellcome). It was not found necessary to use any control measures against ectoparasites.

(2) Cattle: All the calves were regularly immunized with inactivated types 'O' and 'A' Foot and Mouth Disease vaccine (Burroughs Wellcome), and with 'Blanthrax'. They were also given Rinderpest tissue culture vaccine, prepared in the E.A.V.R.O. laboratories. In order to prevent the spread of tick-borne diseases, which are very common in Kenya, all the cattle were dipped weekly in 'Altik' (Cooper, McDougall and Robertson (East Africa).

Disease treatment and control

A. Anaplasmosis and babesiasis

Twelve weeks after arrival one of the calves developed clinical symptoms of Anaplasma marginale infection which responded to treatment with oxytetracycline ('Terramycin', Pfizer). However, it was considered that the disease should be eradicated from the experimental herd, and its reintroduction prevented, before the calves were infected with

F. gigantica. Serum was collected from all the animals and Mr. J. P. J. Ross carried out capillary tube agglutination tests (C. A. tests), for evidence of A. marginale and Babesia bigemina infections. For the former parasite he used the test described by Ristic (1962), as modified by himself and Löhr, the antigen being prepared from local strains of the organism (Ross, personal communication, 1967). The test for B. bigemina infection was that described by Löhr and Ross (1969). Two more calves gave a positive reaction to the test for A. marginale and two to that for B. bigemina, one of these being the calf with clinical anaplasmosis. These infected animals were removed to a separate pasture. All the calves were given therapeutic doses of oxytetracycline ('Terramycin', Pfizer), followed after two days by treatment with 'Berenil' (Hoechst). These treatments were given to try to eliminate incubating infections. Thereafter, the C. A. tests for both diseases were carried out every month by Ross until the end of the experiments.

No further infected animals were found and it would thus appear that these measures, together with weekly immersion in 'Altik' at the recommended strength, and the provision of separate housing and pastures gave satisfactory control of these diseases. Roby (1967) described how A. marginale infection was apparently eradicated from a heavily infected herd in Texas, using the complement fixation test which Ristic (1962) had

found comparable to his C.A. test. The fact that biting insects are not common at E.A.V.R.O. may have helped in the control of these diseases. A separate sterile needle was, of course, used for each animal for all blood sampling and injections.

B. Infections with Eperythrozoon sp.

These were observed in 8 calves at E.A.V.R.O. In 6 of these the parasites were seen in blood smears on one occasion only, and the only apparent effect was a fall in the total leucocyte count, which had returned to its previous level when the next routine examination was carried out a week later. However, in B509 the infection was associated with a fall in the packed cell volume (P.C.V.) from a level of 29½% to 20%. The previous level was only regained after about 3 weeks although the parasites were only seen in blood smears on one day. None of these animals received any treatment. However, B502, with marked symptoms of fascioliasis, also developed an infection with Eperythrozoon. When blood smears taken the following day also showed the infection, this animal was treated with 'Spirotrypan forte' (Hoechst). Only half the recommended dose was given because of possible toxic effects on the liver already damaged by Fasciola. Further reference will be made to this in the discussion of the haematological findings.

C. Respiratory infections

(1) Sheep: Some of the sheep in the

experiment described in Chapter 10 developed slight respiratory infections which were treated with 'Mylipen' and 'Dimycin' (Glaxo). Also a few of those at E.A.V.R.O. had occasional nasal discharges which required no treatment.

(2) Cattle: Most of the calves showed symptoms of mild upper respiratory infection which usually resolved without treatment. Some of these were examined by Mr. M. Gitter who was unable to isolate any pathogenic bacteria. Where treatment was indicated oxytetracycline ('Terramycin', Pfizer) was used.

The possible effect of these infections on the total and differential leucocyte counts will be referred to later.

D. Control of gastro-intestinal nematodes

(1) Sheep: The control of these parasites in the housed sheep in Edinburgh has already been described. At E.A.V.R.O. the sheep, which were out grazing every day, were regularly treated with thiabendazole at 50 mg. per kg. live-weight. They were treated on three occasions after infection with F. gigantica. It had previously been ascertained that the drug is ineffective against F. hepatica (Green, 1967, personal communication), and that it has no action against Fasciola spp. (Gibson, 1967, personal communication).

(2) Cattle: All the calves were dosed on

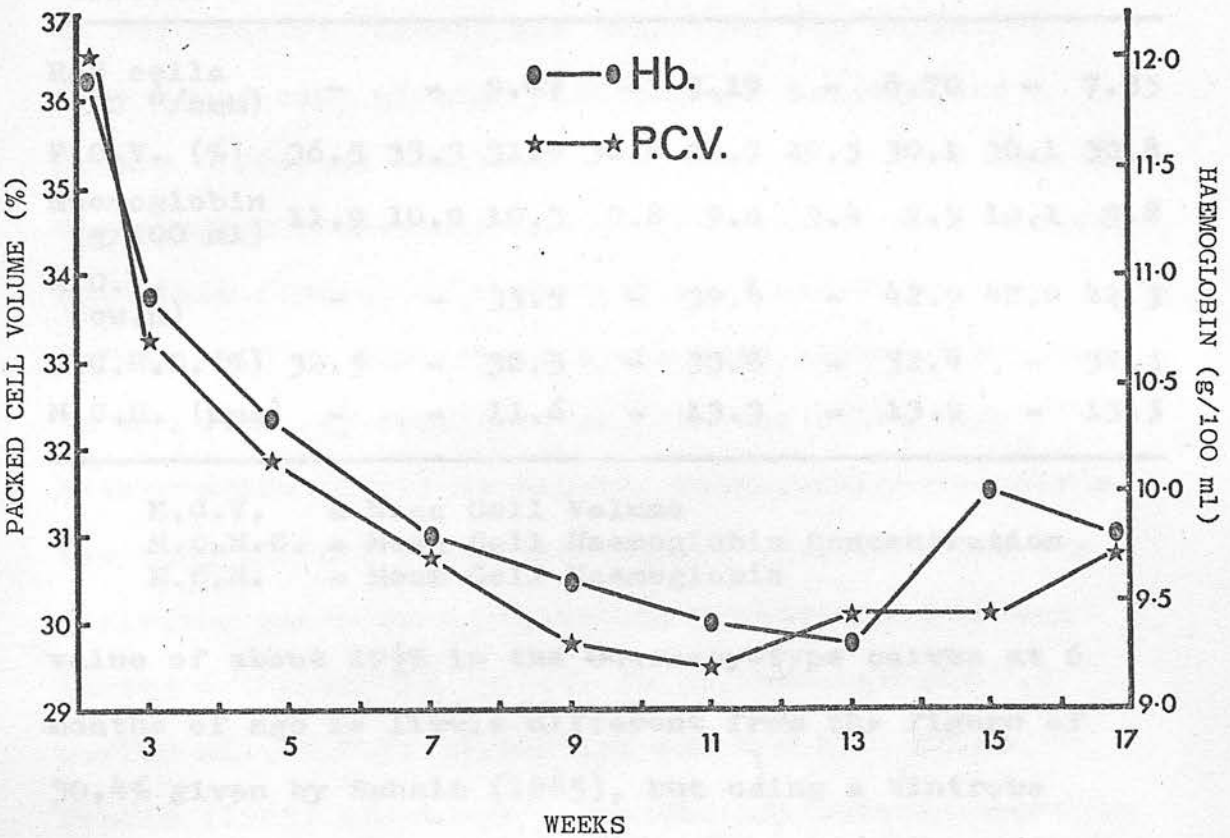
two occasions with thiabendazole at 110 mg. per kg. live-weight, before they were infected with F. gigantica. 'Nilverm' (Imperial Chemical Industries) was substituted for the second dose in the younger calves after Dictyocaulus viviparus infection had been found.

E. Control of lungworms

After D. viviparus infection had been diagnosed clinically, all the calves were treated with 'Franocid' (Burroughs Wellcome). This was before infection with F. gigantica. Dictyocaulus larvae were not found in the faeces, but a few specimens were found at autopsy some months later.

An investigation into the aetiology of the fall in the P.C.V.s of the uninfected calves

The 16 calves, which comprised the first experimental herd, arrived at E.A.V.R.O. on 7th July 1967, when they were 3 to 4 months old. Details of their feeding and husbandry subsequent to this have already been given. They came from a farm of slightly lower altitude where the temperatures were rather higher. A haematological examination was carried out on all the calves at weekly intervals, beginning a fortnight after arrival. The means of some of the indices are given in Graph 8.1 and Table 8.3. Over the initial period of 6 weeks the mean P.C.V. fell from 36.5% to 30.8%, after which it only fell a further 1.3% over the next 4 weeks to reach its lowest point. It then began to rise again slowly. The lowest mean



GRAPH 8.1 HAEMATOLOGICAL VALUES OF UNINFECTED CALVES (GROUP 7-7-67)

Table 8.3

Selected haematological data from uninfected calves

(Group 7-7-67)

Weeks after arrival	2	3	5	7	9	11	13	15	17
Red cells (10^6/cmm)	-	-	9.07	-	7.19	-	6.70	-	7.35
P.C.V. (%)	36.5	33.3	31.9	30.8	29.7	29.5	30.1	30.1	30.8
Haemoglobin (g/100 ml)	11.9	10.9	10.3	9.8	9.6	9.4	9.3	10.1	9.8
M.C.V. (cu.μ)	-	-	35.5	-	39.4	-	42.9	42.0	42.3
M.C.H.C.(%)	32.5	-	32.5	-	33.6	-	32.4	-	32.5
M.C.H. (μμg)	-	-	11.6	-	13.3	-	13.9	-	13.5

M.C.V. = Mean Cell Volume
M.C.H.C. = Mean Cell Haemoglobin Concentration
M.C.H. = Mean Cell Haemoglobin

value of about 29½% in the Guernsey-type calves at 6 months of age is little different from the figure of 30.4% given by Schalm (1965), but using a Wintrobe haematocrit, for Jersey females 7½ to 9 months old, the M.C.H.C. is also little different from his 33.2% but the M.C.V. is markedly different to his 28.5 cu.μ. However, Holman (1956), for Ayrshire females of 6 months old, gave a mean M.C.V. of 40.8 cu.μ. which is near those recorded here. His mean M.C.H.C. of 31.8% is also near the means found in the Guernsey-type calves, but his mean P.C.V. of 34.8% is higher. He also showed a steady increase in the M.C.V. from the

age of 2 months to 16 months although this was not quite so marked as in Table 8.3. Although their figures are for females, Holman and Dew (1967) considered that for all practical purposes steers and heifers, under the same conditions of environment, could be accepted as having the same blood picture. No comparative figures are available for calves of this breed kept at high altitudes. Lampkin and Howard (1962) at E.A.V.R.O. reported a gradual fall in the average haemoglobin levels in suckled zebu calves, but their finding of a mean of 13.5 g./100 ml. for calves of 3 to 6 months old was much higher than that in the Guernsey-type calves. On the other hand Bitakaramire (1969) at Kabete, Kenya, which is only a few hundred feet lower than E.A.V.R.O., reported the following means from his housed 8 months old calves (breed not stated): P.C.V. 30.3%, haemoglobin 7.8 g./100 ml., M.C.V. 37.0 cu.μ. and M.C.H.C. 25.6%. Schalm (1965) considered that the hypochromic anaemias are characterized by the M.C.H.C. falling below 30%.

Although the calves appeared to be rather thin their weight gains were satisfactory, and there were no clinical signs of disease. As a mineral supplement was fed any such deficiency was very unlikely. Blood copper values of three animals (1.03, 0.87 and 1.11 parts per million of copper) ruled out a deficiency of the mineral as a cause of the low haemoglobin levels. These analyses were carried out by Dr. D. A. Howard.

A deficiency of iron is unlikely in grazing stock in Kenya because of the large quantities present in the soil and therefore in dust on herbage (Howard, 1963).

Blood films were examined weekly from all the calves and no abnormalities nor parasites were observed. The C.A. test for both A. marginale and B. bigemina was also negative in all the animals included in the investigation. Those calves which were positive to either of the C.A. tests have been excluded from the results in Table 8.3 and Graph 8.1.

Faeces from all the calves were examined weekly, only low nematode egg counts being recorded.

Conclusion

The falls in the mean P.C.V.s and haemoglobin levels and the rise in the M.C.V.s were probably physiological and related to the age of the calves. They may have been accentuated by the cold damp weather at E.A.V.R.O. at that time of the year, together with a change of feeding and management. The repeated blood sampling may also have affected these values, until the calves became conditioned to being bled, as has been shown by Gartner, Callow, Granzien and Pepper (1969).

Only after the values had stabilized were the experimental infections carried out.

SECTION IV

Results and discussion of experimental infections

Chapter 9 — Acute fascioliasis in sheep (*Fasciola hepatica*)

Chapter 10 — Chronic fascioliasis in sheep (*Fasciola hepatica*)

Chapter 11 — Chronic fascioliasis in sheep (*Fasciola gigantica*)

Chapter 12 — Chronic fascioliasis in cattle (*Fasciola gigantica*): Single infections

Chapter 13 — Chronic fascioliasis in cattle (*Fasciola gigantica*): Single and challenge infections

Chapter 14 — Prolonged chronic and other infections in cattle (*Fasciola gigantica*)

Chapter 15 — The gross pathology of *Fasciola gigantica* infections in cattle

Chapter 16 — Discussion of experimental infections

CHAPTER 9

Acute Fascioliasis in Sheep (Fasciola hepatica)

Introduction

The pathogenesis of acute fascioliasis in sheep was studied in one experiment. At the time this work was carried out (1965) there had been few reports in the literature of such experimental studies.

The literature on acute fascioliasis has been reviewed in Chapter 3.

Experimental design

(a) Animals

Five adult Blackface wethers were obtained from a farm with no history of fascioliasis. The faeces of all animals were checked for Fasciola eggs before the start of the experiment. The sheep were kept indoors in pens with concrete floors and their management and feeding have been described in Chapter 8. Wood shavings were used for bedding and the pens were cleaned weekly to minimise re-infection with gastro-intestinal nematodes.

(b) Treatments

The animals to be infected were selected at random; 2 sheep (11 and 14) were each given 5,500 metacercariae and 2 (12 and 13) were each given 11,000. One sheep (15) was used as an uninfected control.

The infections were carried out by Dr. M. M. H. Sewell who also supplied the initial data.

(c) Observations

Packed cell volumes (P.C.V.s), red cell, eosinophil and total leucocyte counts and haemoglobin estimations were determined weekly. Differential leucocyte counts were made at irregular intervals.

Serum was collected weekly for use in the biochemical studies. The bromsulphthalein dye excretion test was only able to be carried out from 4 weeks after infection.

F. hepatica egg counts were carried out at irregular intervals on faecal samples from the one sheep in which the infection became patent.

The sheep were weighed at weekly intervals.

Results

1. Clinical data

Up to a month after infection there was little to distinguish the infected animals from the controls. Thereafter the infected sheep rapidly developed clinical signs of anaemia, and became progressively weaker, listless and with partial inappetance during the weeks before death. Sheep 12 showed a marked jaundice on the day prior to death; this was the only sheep to show this symptom.

Patches of wool became detached from all the infected sheep during the later stages of the disease.

Sheep 14 seemed to be improving clinically 13 weeks after infection. However, after sub-maxillary

oedema developed during the next week, the condition of this animal deteriorated rapidly and it died 4 days later. This sheep had constipation for the last 3 weeks of its life.

Those animals which lived the longest were in the poorest bodily condition.

The changes in live-weight of the sheep are shown in Table 9.1.

The uninfected control (No. 15) gained weight for 8 weeks after which it fluctuated at about this level. Sheep 14 also showed a gain in live-weight until 10 weeks after infection. However, the other 3 infected sheep only fluctuated around their initial weights during the first part of the experiment. All the infected sheep lost weight during the few weeks before death.

2. Parasitological data

The only infection to become patent was that of sheep 14. The prepatent period was not determined but there were 104 eggs per gram of faeces (e.p.g.) at 80 days, 417 e.p.g. at 89 days, 1112 e.p.g. at 100 days and 1872 e.p.g. at death at 104 days post-infection. As previously stated, this animal was very constipated terminally.

Other data are given in Table 9.2. The percentage recovery is defined as the number of flukes recovered expressed as a percentage of the number of metacercariae administered to the sheep.

Table 9.1

Changes in live-weight of sheep (lbs)

Sheep No.	Initial Weight	Change in live-weight at weeks													
		1	2	3	4	5	7	8	9	10	11	12	13	14	16
11	66	-1	-2	0	-1	-3	-1	+3	-2	-4	-6				
12	78	+2	0	+1	0	-1	-3	-7							
13	92	+2	+1	+1	+2	+4	+2	+4	0	-6	-17				
14	73	+1	0	+3	0	+3	+4	+11	+4	-1	-7	-9	-10	-10	-8
15	67	+6	+5	+7	+9	+12	+14	+20	+17	+16	+13	+19	+19	+19	+18

3. Post-mortem examination

Some of the results of the post-mortem examinations are shown in Table 9.2.

Table 9.2

Some post-mortem details

Sheep No.	11	12	13	14	15
Days from infection to death	72	57	77	104	Control
Infective dose of metacercariae	5,500	11,000	11,000	5,500	0
Wt. of liver (g.)	830	-	1,575	725	-
Nos. of *F. hepatica* recovered	856	-	2,292	1,016	0
% recovery	15.6	-	20.8	18.5	0
Dry weight per fluke (mg)	-	-	-	6.21	0
F. hepatica eggs in gall bladder	Absent	Absent	Absent	Present	Absent

All the infected sheep died of fascioliasis. The lesions were similar to those described by Pullan (1968) in acute and sub-acute fascioliasis in sheep and so will only be described briefly.

The liver of sheep 12 was enlarged and had an orange-brown mottled appearance with prominent haemorrhagic tracks. The gall bladder was very enlarged and black in colour. Petechial haemorrhages were prominent in the kidneys.

The bile of sheep 11 was nearly black in colour and the gall bladder enlarged. The liver was also

enlarged, but paler in colour than that of sheep 12, and the haemorrhagic tracks were again very prominent. The lesions found in sheep 13 were very similar to these, together with prominent areas of fibrinous peritonitis on the surface of the liver.

Sheep 14 had lesions of severe chronic fascioliasis with the large bile ducts distended with liver flukes. No lesions were found in any organ when the control animal, No. 15, was slaughtered at the conclusion of the experiment.

The peritoneal cavities of these sheep which died in the acute phase of the disease contained large quantities of serosanguinous fluid.

Two immature flukes were present in the gall bladder and one in the main hepatic bile duct of sheep 11. There were also two immature flukes in the gall bladder of sheep 13, 8 and 10 mm. long respectively. The lungs of this sheep showed large areas of congestion, and sections showed that these areas contained numerous typical haemorrhagic tracks due to migrating flukes. In one section there was a Fasciola in a subpleural position and subpleural oedema was prominent.

One immature fluke was also found in the gall bladder of sheep 12.

The livers of the infected sheep were considerably enlarged, especially that of No. 13. A direct comparison could not be made with the liver weight of

the control, No. 15, as this was not available. However normal livers from sheep of similar live-weight had a range of 400-450 gm. (Chapter 11).

The carcass of sheep 12 was severely jaundiced - the only one to show this lesion.

4. Biochemical data

(a) Bromsulphthalein (B.S.P.) dye excretion test: The test results are shown in Table 9.3.

Table 9.3

B.S.P. half clearance times from plasma (mins)

Sheep No.	Weeks post-infection											
	4	5	6	7	8	9	10	11	12	13	14	15
11	4.8	-	4.5	-	6.0	9.8	15.0					
12	5.1	6.2	9.4	19.3								
13	3.5	3.3	4.6	7.2	13.1	13.7	13.0					
14	4.4	-	3.0	-	4.6	5.5	5.8	3.9	3.3	3.8	7.4	
15	2.0	-	1.9	-	2.1	2.3	-	2.6	2.1	2.9	-	1.7

The plasma half clearance time was uniformly very brief in No. 15, the uninfected control sheep, but there was a big increase in the 3 animals which died between 57 and 77 days after infection. Sheep 11 died during the test before the 4th, and last, blood sample could be collected and this may have affected the result. The increase in the plasma half clearance time was not so marked in the case of sheep 14 which survived the acute phase of the disease. The longest time recorded in this animal was in the test carried

out a few hours before death. As in the case of sheep 11, this may have been because of terminal circulatory failure.

(b) Iodine flocculation test: The test was carried out on sera stored for 12-18 months at -20°C and interpreted as described by Hindson (1965), except that one drop of reagent was added to 1, 2, 4 and 8 drops of serum consecutively (instead of to ½, 1, 2 and 4 drops). The results are shown in Table 9.4.

Table 9.4

Results of iodine flocculation test

Sheep No.	Weeks post-infection														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
11	0	1	4	16	16	12	16	12	16	16					
12	0	4	8	16	12	12	16	16							
13	0	0	0	4	8	8	16	16	24	24	24				
14	0	0	1	4	4	4	4	8	-	16	12	16	16	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

0 = negative. The amount of flocculation has been scored within a scale 1-4. The figures shown are derived by multiplying this score by the number of drops of serum used. All sera taken preinfection were negative.

While the control sheep (No. 15) remained negative to this test, all the infected ones became positive by 4 weeks after infection. Further, the two sheep which died first gave a positive reaction as early as 2 weeks

post-infection. All the infected sheep remained strongly positive until death, except for No. 14 which was negative at all dilutions after 13 weeks post-infection.

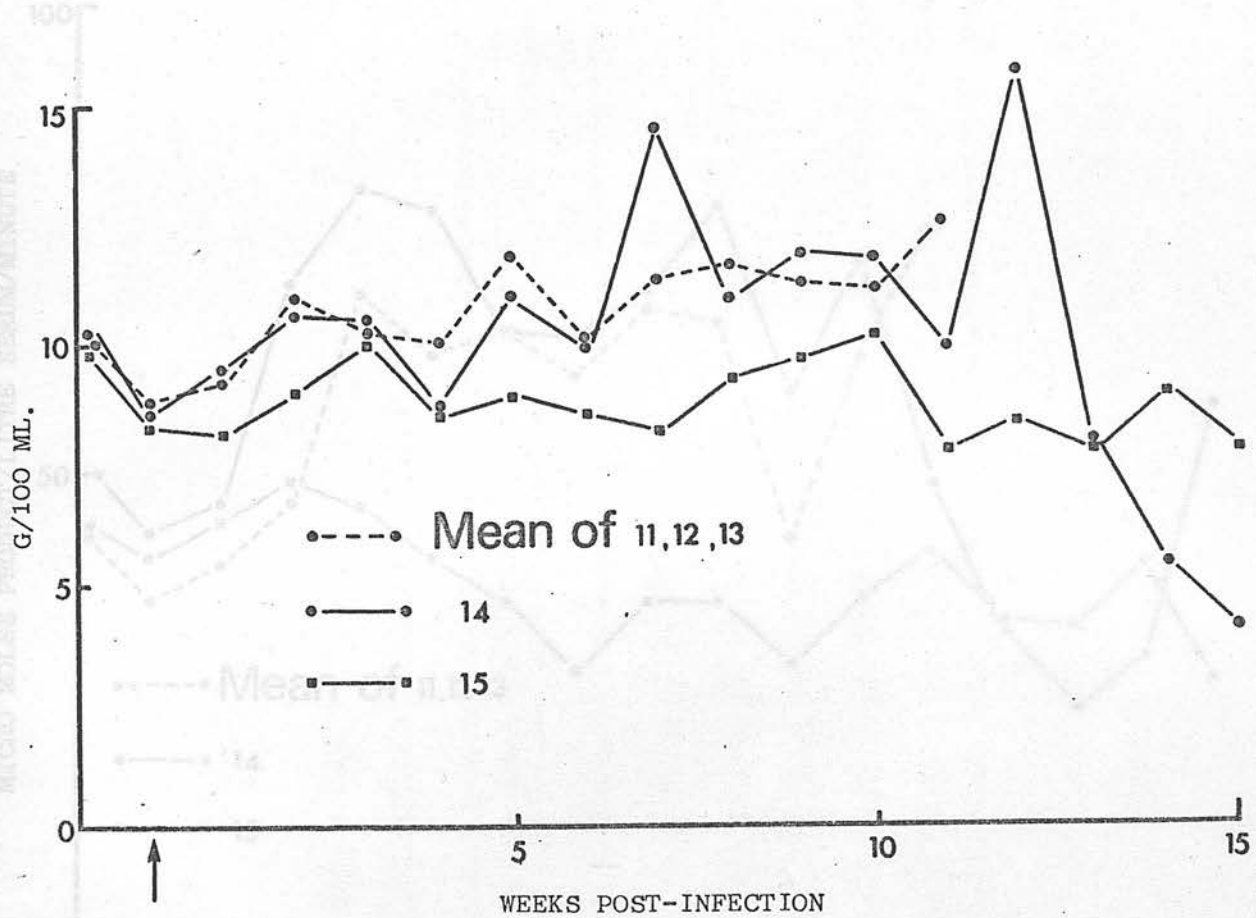
(c) Total serum protein: The results of the total serum protein estimations are shown in Table 9.5 and Graph 9.1. In the graph the means of the values for sheep 11, 12 and 13 are given for clarity.

The uninfected control (No. 15) maintained a fairly uniform level of total serum protein throughout the experiment. However all the infected sheep had slightly elevated levels in the acute phase of the infection, although these were rather variable. The concentrations in sheep 14 fell terminally to half the preinfection level.

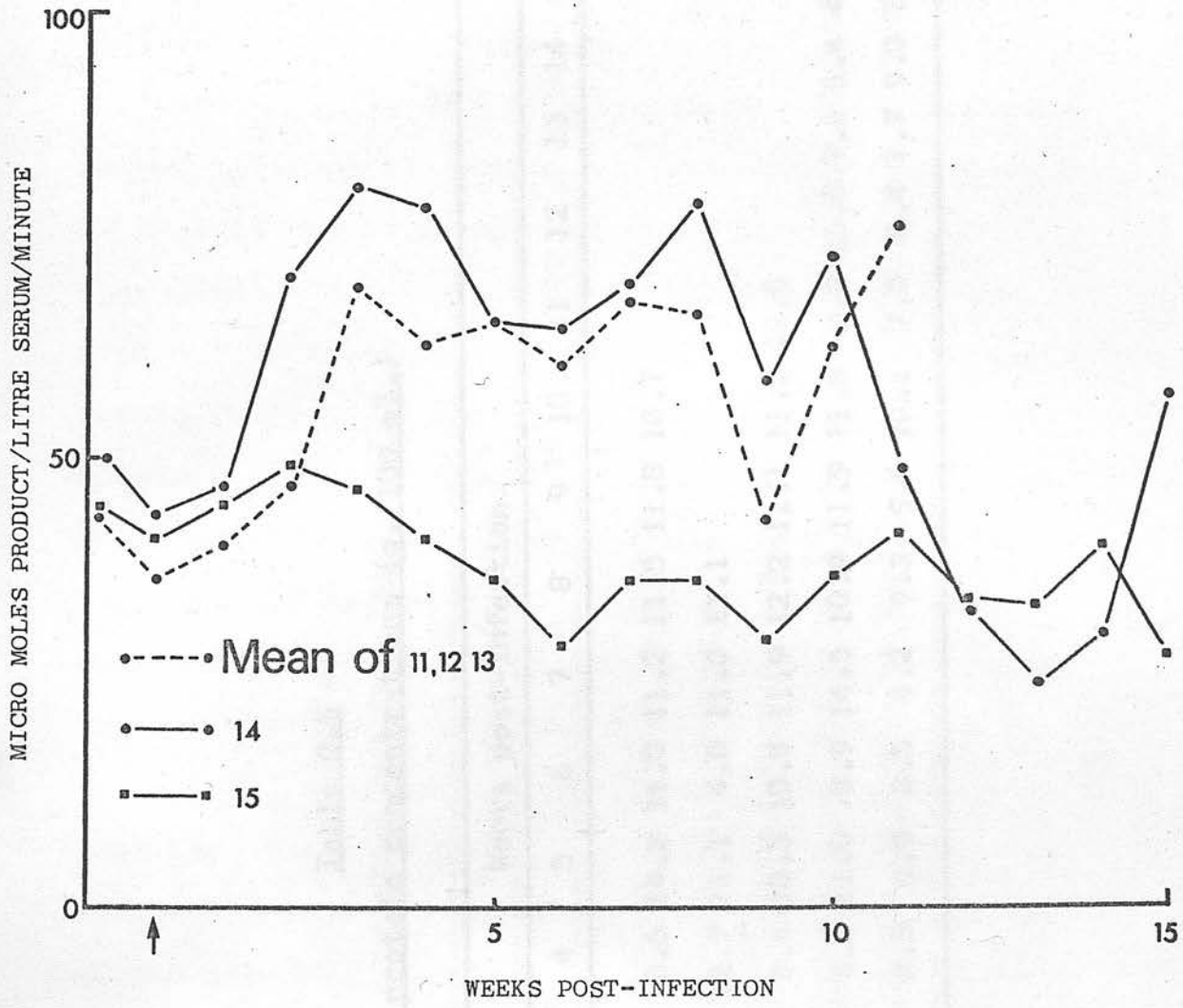
(d) Serum glutamic pyruvic transaminase (S.G.P.T.): The results of the weekly assays for S.G.P.T. activity are not recorded as there were no significant changes during the experimental period in any sheep.

(e) Serum glutamic oxaloacetic transaminase (S.G.O.T.): The results of the G.O.T. estimations are shown in Table 9.6 and Graph 9.2.

The values for the uninfected control (No. 15) remained at a uniformly low level, but those of all the infected sheep showed a rise which commenced in most cases two weeks after infection. From three weeks until eight weeks after infection the values remained



GRAPH 9.1 TOTAL SERUM PROTEIN



GRAPH 9.2 SERUM GLUTAMIC OXALOACETIC TRANSAMINASE

Table 9.5

Total serum protein concentrations (g./100 ml.)

Sheep No.	Preinfection		Weeks post-infection														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
11	10.0	9.2	8.8	12.6	9.3	8.6	14.2	11.0	11.2	11.5	11.8	10.7					
12	10.0	9.2	9.7	10.6	10.1	12.9	11.1	4.8	11.0	11.1							
13	10.3	8.2	9.2	9.9	11.4	8.4	10.3	10.8	11.9	12.2	11.1	11.5	12.6				
14	10.3	8.6	9.5	10.7	10.4	8.5	11.0	8.9	14.5	10.9	11.9	11.8	9.9	15.6	7.8	5.4	4.0
15	10.0	8.4	8.2	9.0	10.0	8.5	8.9	8.5	8.2	9.3	9.6	10.1	7.8	8.4	7.7	9.0	7.7

Table 9.6

S.G.O.T. concentrations (μ moles/litre/min.)

Sheep No.	Preinfection		Weeks post-infection														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
11	43.6	38.4	46.4	46.8	58.7	51.5	58.3	42.4	46.2	55.3	37.1	49.2					
12	42.9	35.0	37.8	50.6	72.0	59.9	63.6	66.7	65.9	64.4							
13	43.6	37.9	37.9	43.0	75.8	66.7	75.0	73.5	91.7	81.1	49.2	75.0	76.5				
14	50.1	43.6	46.8	70.4	80.5	78.0	65.6	64.3	69.7	78.8	59.1	72.8	49.2	33.3	25.0	31.0	57.6
15	44.9	41.7	44.9	48.7	46.4	41.7	37.1	29.2	37.1	37.1	29.5	37.9	41.7	34.1	34.1	41.7	29.9

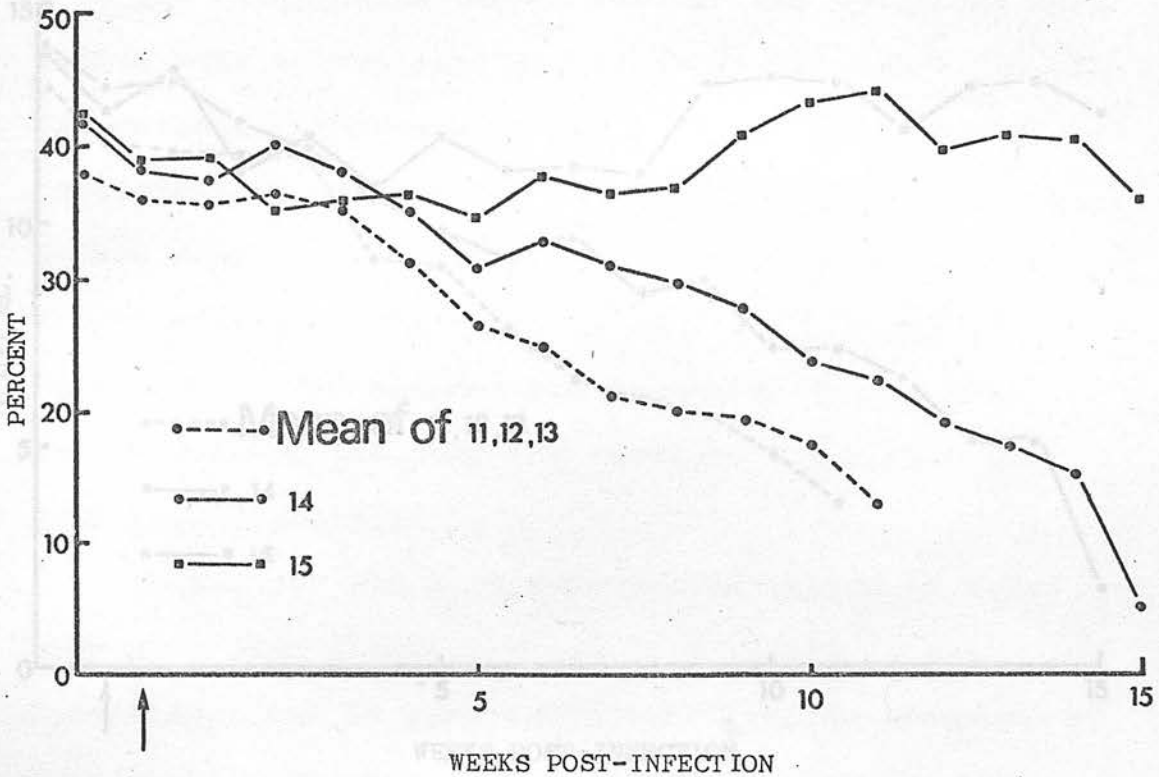
uniformly high, but there was then a variable tendency for them to fall. Sheep 11 however showed a smaller rise than the other infected animals, and this was followed by an earlier fall.

Sheep 13, 14 and to a lesser extent 11, showed a marked terminal rise in G.O.T. activity. In the case of Nos. 13 and 11 nearly all the flukes were still migrating in the liver parenchyma at this time and high G.O.T. levels were expected. In any case these levels followed a steep fall in each case and were merely a return to those previously found. On the other hand the last serum from sheep 14 was collected very shortly before death and it is possible that anorexia resulting from terminal partial circulatory failure had led to the breakdown of tissues rich in the enzyme, thus raising the level in the serum. Boyd (1962) found high G.O.T. activity in cardiac and skeletal muscle, in the kidneys and in brain, as well as the liver of sheep.

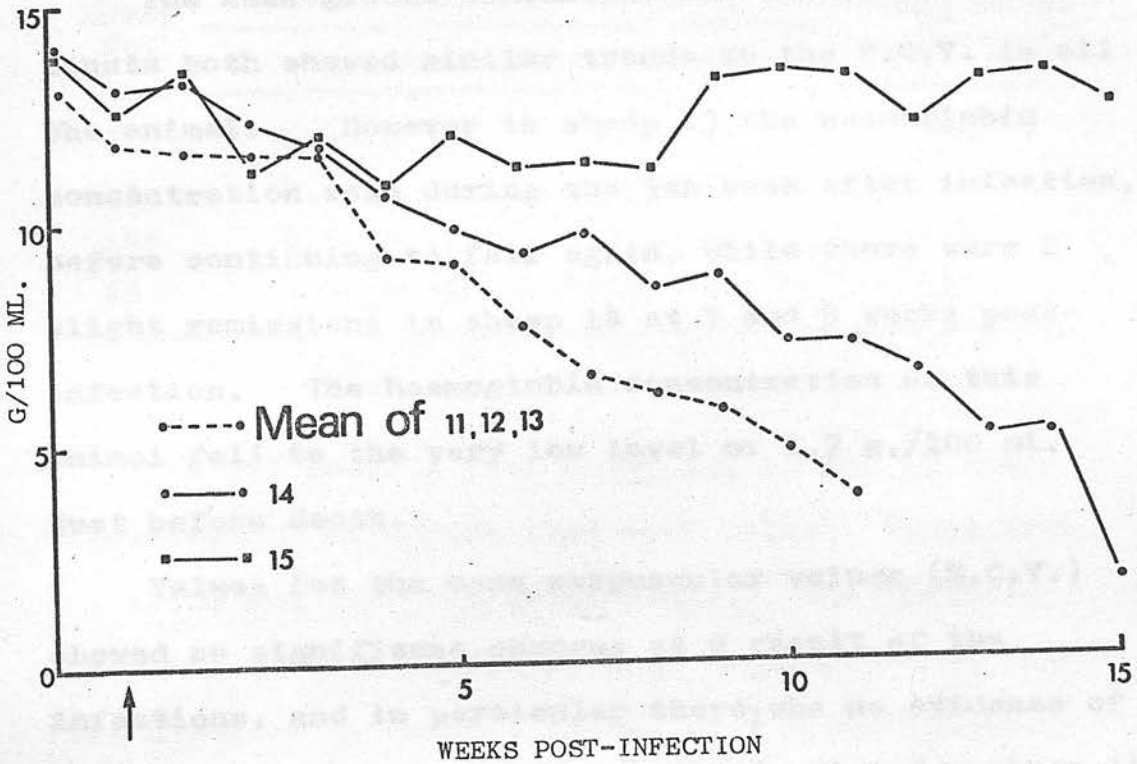
5. Haematological data

The haematological examinations were carried out at weekly intervals and the results for the erythrocyte series are given in Appendix Table 9.A. The P.C.V.s are also shown in Graph 9.3 and the haemoglobin values in Graph 9.4. Again in these graphs, for clarity, the mean values are given for the 3 sheep which died before the infection became patent.

All the infected animals showed a drop in P.C.V.



GRAPH 9.3 PACKED CELL VOLUME



GRAPH 9.4 HAEMOGLOBIN

beginning 2 to 4 weeks after infection, while the control (No. 15) maintained its original level. The falls in the infected animals were progressive, with only a very slight remission in sheep 14 at 6 weeks post-infection. Just before death, the P.C.V. of this animal had fallen to the very low level of 5½%.

The haemoglobin concentrations and red blood cell counts both showed similar trends to the P.C.V. in all the animals. However in sheep 13 the haemoglobin concentration rose during the 5th week after infection, before continuing to fall again, while there were 2 slight remissions in sheep 14 at 7 and 9 weeks post-infection. The haemoglobin concentration of this animal fell to the very low level of 1.7 g./100 ml. just before death.

Values for the mean corpuscular volume (M.C.V.) showed no significant changes as a result of the infections, and in particular there was no evidence of a macrocytic anaemia. The terminal value for sheep 14 was rather low, which would indicate a microcytic anaemia, but at this stage poikilocytosis was very marked so the interpretation of this low value is difficult. The control sheep (No. 15) also showed a fall in M.C.V. during the last 5 weeks of the experiment, although this was not so marked as in the case of the terminal value for sheep 14.

In order to study further the size of the erythrocytes in this anaemia, red cell diameters were

measured and Price-Jones curves were drawn as described by Dacie (1956). The results are shown in Table 9.7.

Table 9.7

Mean red cell diameter (μ)±s.d.

Sheep No.	Mean red cell diameter(μ)±s.d.		Weeks Post-infection
	Preinfection	Post-infection	
11	4.29±0.33	4.44±0.42	10
13	4.43±0.39	4.84±0.50	10
14*	4.62±0.41	4.66±0.48	14
15	4.62±0.36	4.32±0.34	15

* Means of two estimations.

The two results from sheep 11, 13 and 15 are significantly different from each other; those from No. 14 do not differ significantly. The infected sheep all show an increased mean cell diameter, while this diameter is reduced in the uninfected animal. However, there are insufficient observations to show whether this difference is real.

The mean corpuscular haemoglobin (M.C.H.) fell slightly, both in the infected and in the control sheep, during the course of the experiment. Again the terminal value for sheep 14 was lower than any other. The mean corpuscular haemoglobin concentration (M.C.H.C.) fell slightly in all the infected animals but not in the control sheep. However, the falls in

these two values were not marked, and the anaemia might be considered as normochromic in sheep 12 and 14 and very lightly hypochromic in 11 and 13 as Schalm (1965) considers that the hypochromic anaemias are characterized by the M.C.H.C. falling below 30%.

Sheep 11, 12 and 13 all had immature erythrocytes in blood smears taken on weeks 9 or 10 after infection and these increased in number until the animals died (Table 9.8).

Table 9.8

Incidence of abnormal erythrocytes

	Sheep No.	Weeks post-infection						
		9	10	11	12	13	14	15
Polychromasia	11	-	+					
	13	+	+	+				
	14	-	+	+	+	+	+	+
Punctate basophilia (%)	11	-	0.05					
	13	0.15	0.55	1.30				
	14	-	0.35	0.75	1.0	1.55	3.0	4.55
Anisocytosis	11	-	-					
	13	+	+	+				
	14	-	-	-	-	-	+	+
Poikilocytosis	11	-	-					
	13	+	+	+				
	14	-	-	+	+	+	+	+

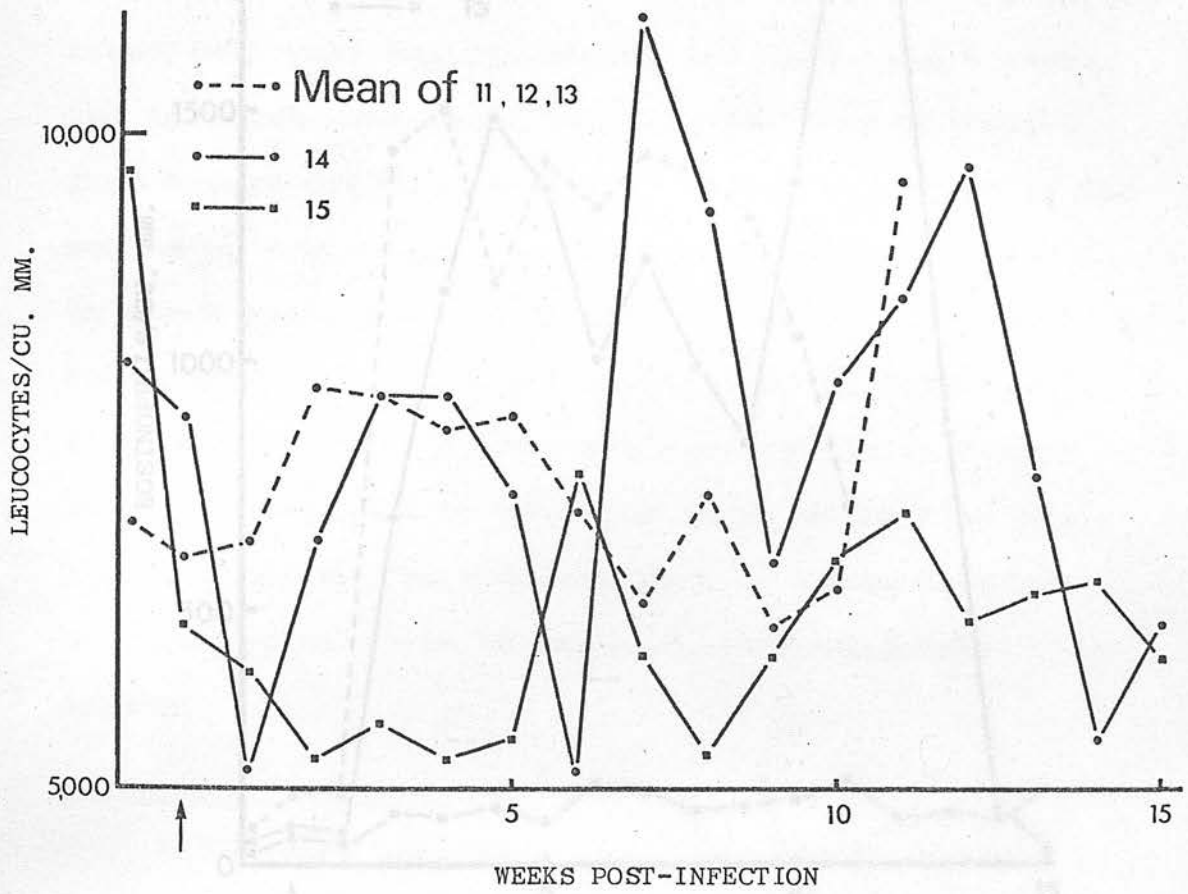
Blood smears from sheep 12 were not available, all from No. 15 were negative as were those from the other sheep until week 9. The examination for evidence of punctate basophilia was based on 2000 erythrocytes. + present, in each case becoming more marked with time until

death. - not present.

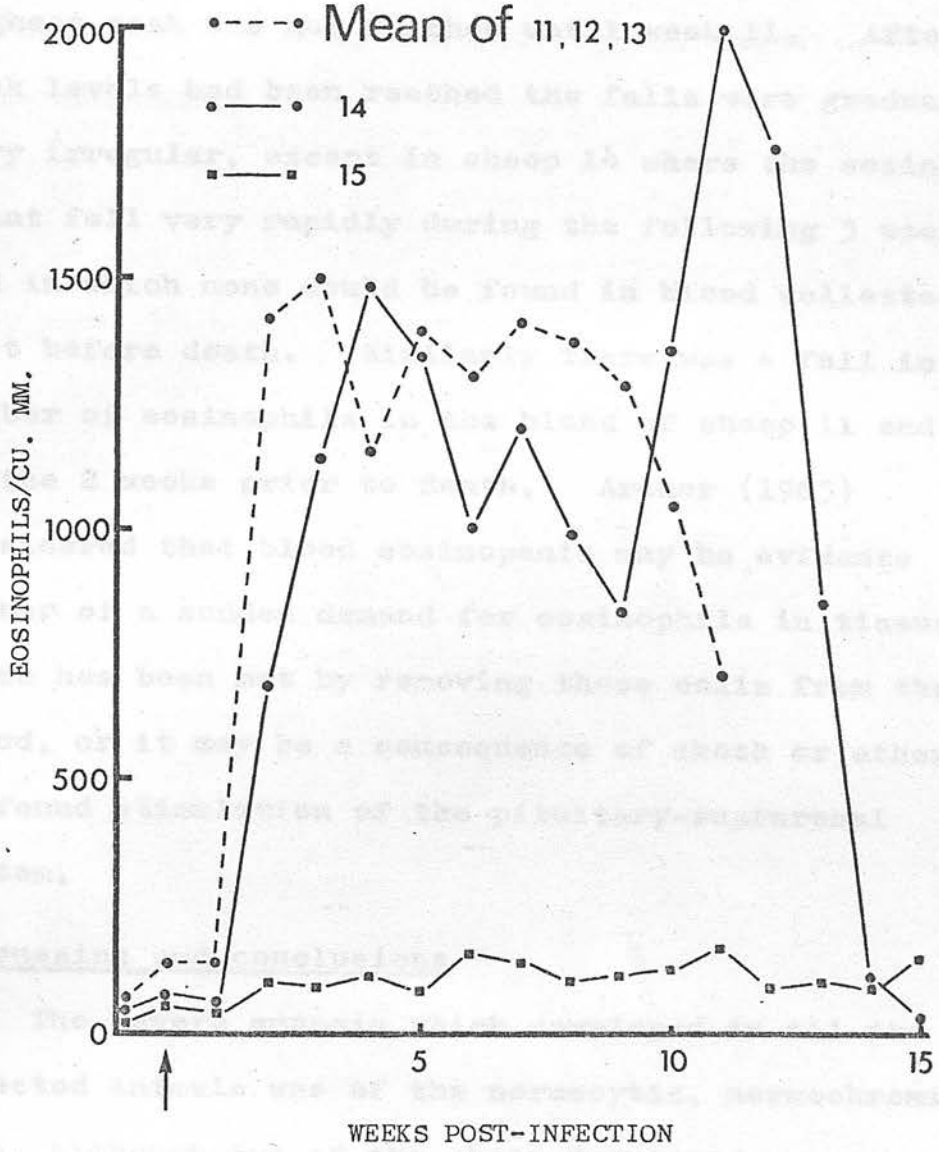
Sheep 14 had the highest level of erythrocytes showing punctate basophilia, and these had reached 4.6% at the time of death. Polychromasia was also recognized at the same times and in the same blood smears as the punctate basophilia. Poikilocytosis and anisocytosis became marked in blood smears from sheep 13 from the 9th week after infection, and in those from sheep 14 from the 11th week (poikilocytosis) and the 14th week (anisocytosis). No nucleated red cells were seen. Blood smears were not available for study from sheep 12, while those from the control, No. 15, had normal morphology and staining affinity throughout the experiment.

The white blood cell results are given in Appendix Table 9.B. The total white cell counts are also shown in Graph 9.5 and the total eosinophil counts in Graph 9.6.

There was a leucocytosis from the 2nd week after infection in most of the sheep, which could be largely accounted for by the increase in eosinophil counts. The mean total white cell counts were at their highest levels 3 weeks after infection, but sheep 14 had further increases at the 7th and 12 weeks. There was also a neutropenia on the 3rd week after infection in sheep 11, which was not seen in Nos. 13 or 14. However blood smears were not available to enable a full study to be carried out in all the sheep.



GRAPH 9.5 TOTAL LEUCOCYTE COUNTS



GRAPH 9.6 EOSINOPHIL COUNTS

The eosinophil counts rose by the 2nd week after infection and reached maximum levels shortly after that, except in the case of sheep 14 where the highest peak was not reached until week 11. After the peak levels had been reached the falls were gradual but very irregular, except in sheep 14 where the eosinophil count fell very rapidly during the following 3 weeks, and in which none could be found in blood collected just before death. Similarly there was a fall in the number of eosinophils in the blood of sheep 11 and 12 in the 2 weeks prior to death. Archer (1963) considered that blood eosinopenia may be evidence either of a sudden demand for eosinophils in tissue which has been met by removing these cells from the blood, or it may be a consequence of shock or other profound stimulation of the pituitary-suprarenal system.

Discussion and conclusions

The severe anaemia which developed in all the infected animals was of the normocytic, normochromic type, although two of the sheep developed a very slight hypochromia.

While the B.S.P. dye excretion test, the iodine flocculation test and the S.G.O.T. assay demonstrated the pathogenesis of the liver disease, the S.G.P.T. assay revealed no significant changes and accordingly it was decided to discontinue the use of this assay in

further experiments. Similarly, measurements of red blood cell diameters and drawing Price-Jones curves was discontinued as it showed little that had not already been shown by the M.C.V., and was also very time consuming.

CHAPTER 10

Chronic Fascioliasis in Sheep
(Fasciola hepatica)

Introduction

Adult F. hepatica have usually been assumed to feed by ingesting blood, thus causing a haemorrhagic type of anaemia. However, some recent studies have suggested that the anaemia may result from a relative dyshaemopoiesis and from a reduction in red cell life (Sinclair, 1964, 1965). During 1967 a series of studies was therefore carried out on the aetiology of the anaemia, and at the same time the peripheral blood leucocyte picture was followed in detail.

Certain other observations, mainly of a parasitological nature, were also made. The literature on chronic fascioliasis has been reviewed in Chapter 3.

Experimental design

(a) Animals

Nine 6 to 7 month old Cheviot wethers were obtained from a farm with no history of fascioliasis and maintained as previously described. The sheep were numbered 1-10 within the experiment, but in the results the equivalent flock (ear tattoo) number is given.

(b) Treatments

At 11-12 months of age three of the sheep (Nos.

17, 20 and 23) were selected at random and each infected with 2400 metacercariae of F. hepatica, three other sheep (Nos. 24, 25 and 30) were similarly selected and infected with 600 metacercariae each, while the remaining three (Nos. 13, 14 and 29) were left as uninfected controls. However sheep No. 29, one of the control animals, later developed an idiopathic anaemia, which was associated with a fall in live-weight and was slaughtered. No macroscopic lesions were found at the post-mortem examination but the bone marrow was severely hypoplastic. All the results of the examinations of this animal have therefore been excluded from the experiment.

The metacercariae which were used to infect sheep Nos. 24, 25 and 30 were 2-3 weeks old, while those used for Nos. 17, 20 and 23 were 6 weeks old.

(c) Observations

Packed cell volumes (P.C.V.s) and eosinophil counts were determined at weekly intervals. Other haematological examinations were carried out at 3-weekly intervals. Direct differential centrifugal flotation (D.D.C.F.) examinations were made at weekly intervals on faeces from all sheep. Similarly Fasciola egg counts were carried out weekly on faecal samples from all infected sheep.

The sheep were weighed at irregular intervals.

Results

1. Clinical data

The only sheep that seemed likely to die of chronic fascioliasis was No. 17 which progressively lost weight and developed a severe anaemia from 14 weeks post-infection. The P.C.V. had fallen to 11% at 20 weeks after infection when this animal was slaughtered. At the same time No. 30 was also slaughtered as it was apparent that the fluke burden was very low.

No obvious symptoms were present in any of the infected sheep during the acute phase of the disease. However during the last few weeks of the experiment the more heavily infected animals became listless and dull and did not thrive as well as the others. The faeces of all sheep appeared normal and constipation was not seen.

The changes in live-weight of the sheep are shown in Table 10.1.

Table 10.1

Changes in live-weights of sheep (lbs.)

Sheep No.	Initial weight	Changes in live-weight at weeks			
		6	13	20/21	30/31
13	78	-4	+12	-	+37
23	90	+6	+16	-	+16
25	88	+10	+25	-	+42
14	84	+6	+22	-	+42
30	81	+11	+23	+44	
20	85	+5	+11	-	+26
17	78	-6	-2	-14	
24	74	+2	+6	-	+20

Both the uninfected controls (Nos. 13 and 14) and Nos. 25 and 30 gained weight at about the same rate throughout the experiment, after an initial set-back in No. 13. Indeed, sheep 30 had the same live-weight gain at 21 weeks after infection as the other three animals had 10 weeks later. Thus it is apparent that low burdens of F. hepatica (44 and 10 respectively) had no effect on live-weight gain under the conditions of this experiment. However No. 24 with a burden of 84 flukes had a live-weight gain of only half that of the former animals, approximately the same as that for No. 20 with 157 flukes and No. 23 with 367 flukes. At each of the three weighings a weight loss was recorded in sheep No. 17 which had a fluke burden of 322.

Some of the sheep had mild respiratory infections with symptoms of coughing, increased respiratory rates and slightly raised body temperatures. However these symptoms were probably not associated with the F. hepatica infection, as no liver fluke lesions were found in the lungs at the post-mortem examinations.

2. Parasitological data

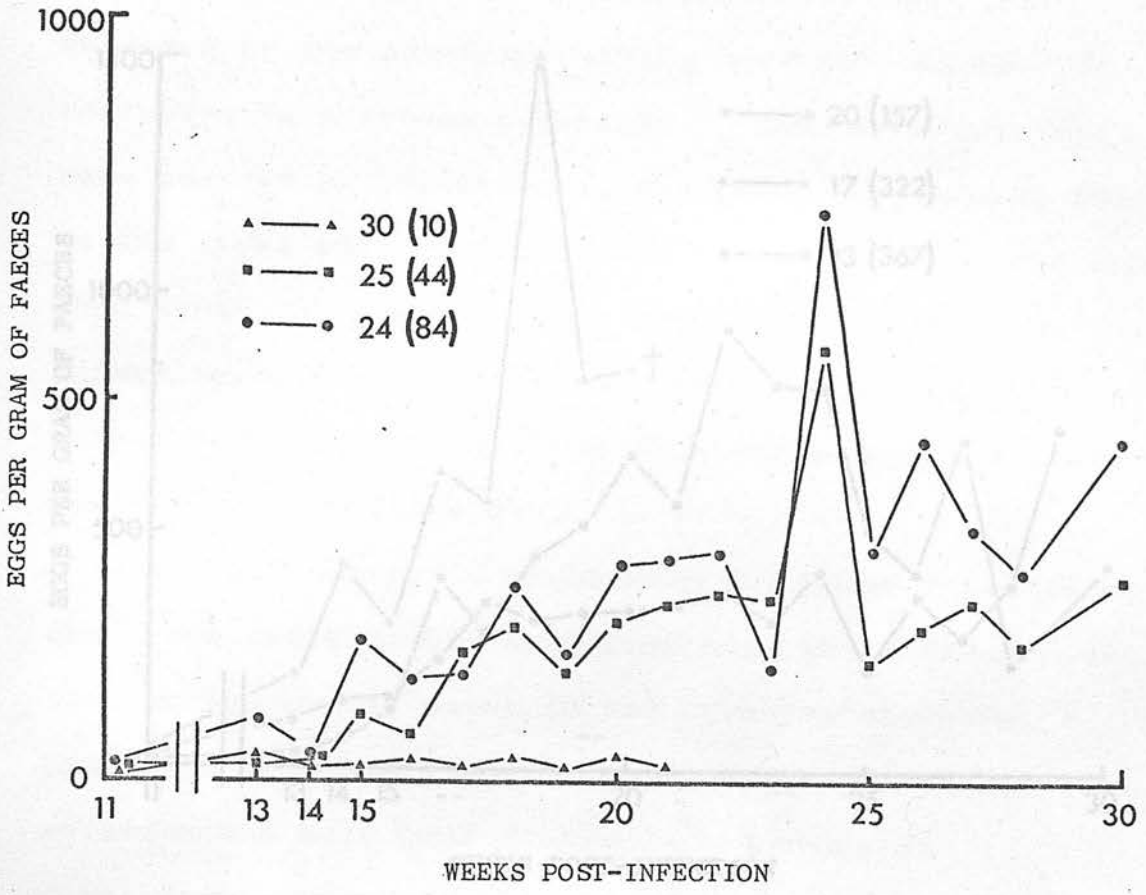
Only very small numbers of coccidial oocysts and occasional Strongyloides and Trichuris eggs were found in some of the sheep in the weekly D.D.C.F. examinations. At the post-mortem examination of each animal the abomasum and the intestines were examined in detail and the virtual absence of nematode infections was confirmed. Very small numbers of Trichuris,

Nematodirus and Haemonchus contortus were found in some of the sheep, Nos. 13, 17, 23, 24 and 30 being apparently free from gastro-intestinal nematodes.

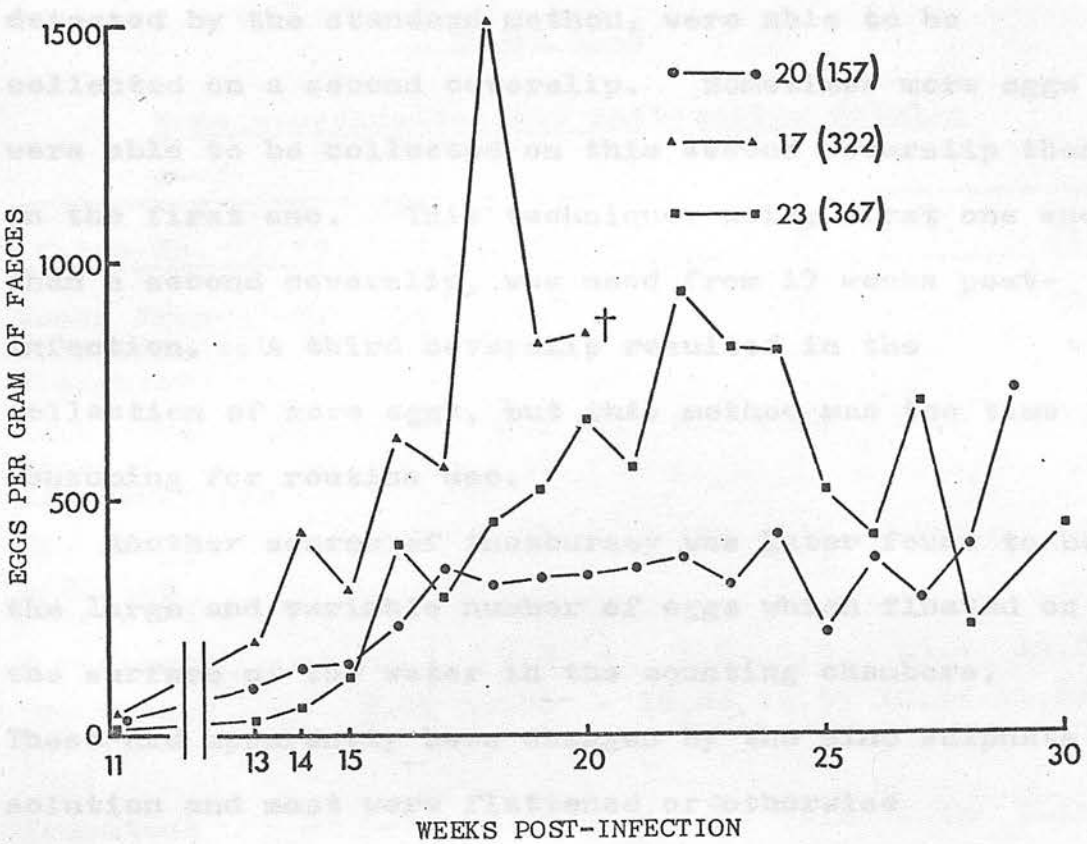
All the sheep which were experimentally infected with F. hepatica developed mature infections. The prepatent periods are recorded in Table 10.2, and the faecal egg counts in Appendix Table 10.A, and Graphs 10.1 and 10.2. The figures in parenthesis on the graphs show the fluke burden in each sheep.

The faecal egg counts tended to increase throughout the experimental period of 30 weeks post-infection, except in No. 23 where the highest count was recorded at 22-24 weeks. Sheep 17 had a much higher count than No. 23 although both had a similar fluke burden. Moreover the pathogenic effects of the infection were much more apparent in the case of No. 17 so that the higher egg count may indicate a lack of resistance by this host.

For the egg counts of week 22 for No. 23, week 23 for No. 20, and weeks 24 and 25 for Nos. 24 and 25, faeces were collected in the morning from the top of the sample in the faecal bags which these sheep were wearing for an experiment using ^{51}Cr (Sewell, Hammond and Dinning, 1968). It is possible that some of the faeces collected had been passed at another time and that these counts were therefore affected by diurnal variation. This may account for the unusually high counts for both Nos. 24 and 25 on week 24.



GRAPH 10.1 FASCIOLA HEPATICA FAECAL EGG COUNTS



GRAPH 10.2 FASCIOLA HEPATICA FAECAL EGG COUNTS

The technique used was always sensitive enough to detect eggs in the faeces of No. 30 which had a fluke burden of only 10.

It will be seen from Appendix Table 10.A that a large and variable number of eggs, which were not detected by the standard method, were able to be collected on a second coverslip. Sometimes more eggs were able to be collected on this second coverslip than on the first one. This technique, using first one and then a second coverslip, was used from 17 weeks post-infection. A third coverslip resulted in the collection of more eggs, but this method was too time consuming for routine use.

Another source of inaccuracy was later found to be the large and variable number of eggs which floated on the surface of the water in the counting chambers. These had apparently been damaged by the zinc sulphate solution and most were flattened or otherwise distorted. They were unable to re-expand in water and had not done so even after 3 days' immersion in one experiment. Dennis, Stone and Swanson (1954) had observed that the operculated shell of F. hepatica prevents the use of high specific gravity flotation techniques as the ova distort in these solutions, and many were observed floating on top of the water thus resulting in inaccurate counts.

It was also noted that the floating eggs would be very difficult to differentiate from paramphistome

eggs, thus making this technique impossible to use in Kenya where these infections are very commonly found in cattle.

For these reasons the use of this method for counting Fasciola eggs in faeces was discontinued.

Table 10.2

Some experimental and post-mortem details

Sheep No.	13	23	25	14	30	20	17	24
Weeks from infection to slaughter	31	30	31	31	21	30	20	30
Infective dose of metacercariae	Nil	2400	600	Nil	600	2400	2400	600
No. of F. hepatica recovered	-	367	44	-	10	157	322	84
% recovery	-	15.3	7.3	-	1.7	6.5	13.4	14.0
Dry weight per fluke (mg)	-	8.69	18.96	-	16.26	15.57	10.75	15.61
Prepatent period (weeks)	-	9-10	10-11	-	11-12	9-10	9-10	9-10
Weight of liver (g) (including gall bladder and lymph nodes)	819	1292	891	759	920	1000	913	922

From Table 10.2 it will be seen that the percentage recovery of flukes was very low when compared with the results of other workers (Boray, 1967; Ross, Dow and Todd, 1967). Possible reasons

for this very low recovery have been discussed in Chapter 6.

In view of the errors inherent in measuring the lengths of liver flukes, the mean dry weights were taken as the measurement of fluke size. This, however, was discontinued when it was found that other workers were consistently using fluke lengths to indicate size, as otherwise there would be no basis for comparison with their results. Furthermore no estimation of the range of flukes sizes can be obtained from the dry-weight method.

While sheep 17 and 23 had comparable fluke burdens the mean dry weight per fluke was appreciably higher at 20 weeks in the former than at 30 weeks post-infection in the latter. Thus F. hepatica was able to grow larger in No. 17 than No. 23. Furthermore the mean dry weight per fluke was only slightly higher in No. 30 with a fluke burden of 10 than in No. 20 with a burden of 157.

3. Post-mortem examinations

The macroscopical lesions of chronic F. hepatica infections in sheep have been described by many authors and so will not be given in detail.

No lesions were found in Nos. 13 and 14 - the uninfected controls. The weights of the livers of all the animals are included in Table 10.2, where the increase in weight with increased liver fluke burden can be clearly seen in those animals which were

slaughtered 30-31 weeks after infection. However, the two sheep which were slaughtered at 20-21 weeks post-infection had livers of almost the same weight, although No. 30 had only 10 and No. 17 had 322 flukes. This was probably related to the size of these animals, because at this time No. 30 weighed 125 lbs but No. 17 only weighed 64 lbs.

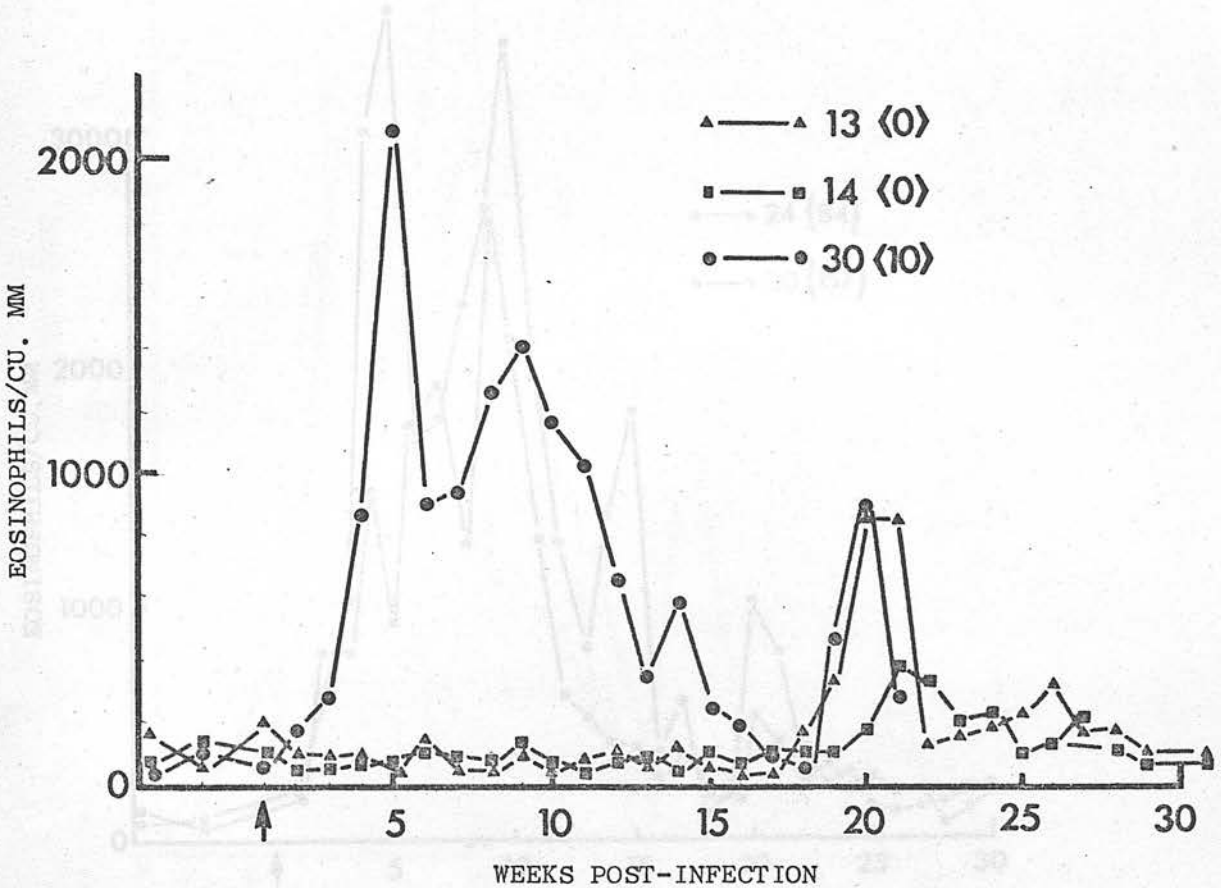
The only other organs in which lesions were found were the lungs of Nos. 24 and 25 which had signs of old localized pneumonia and pleurisy. There were no signs of any residual lesions from the mild respiratory infections which had been seen in Nos. 13, 23 and 30.

Seventeen adult F. hepatica were found in the small intestine of No. 23, 10 in the small intestine of No. 24, and 7 in that of No. 20.

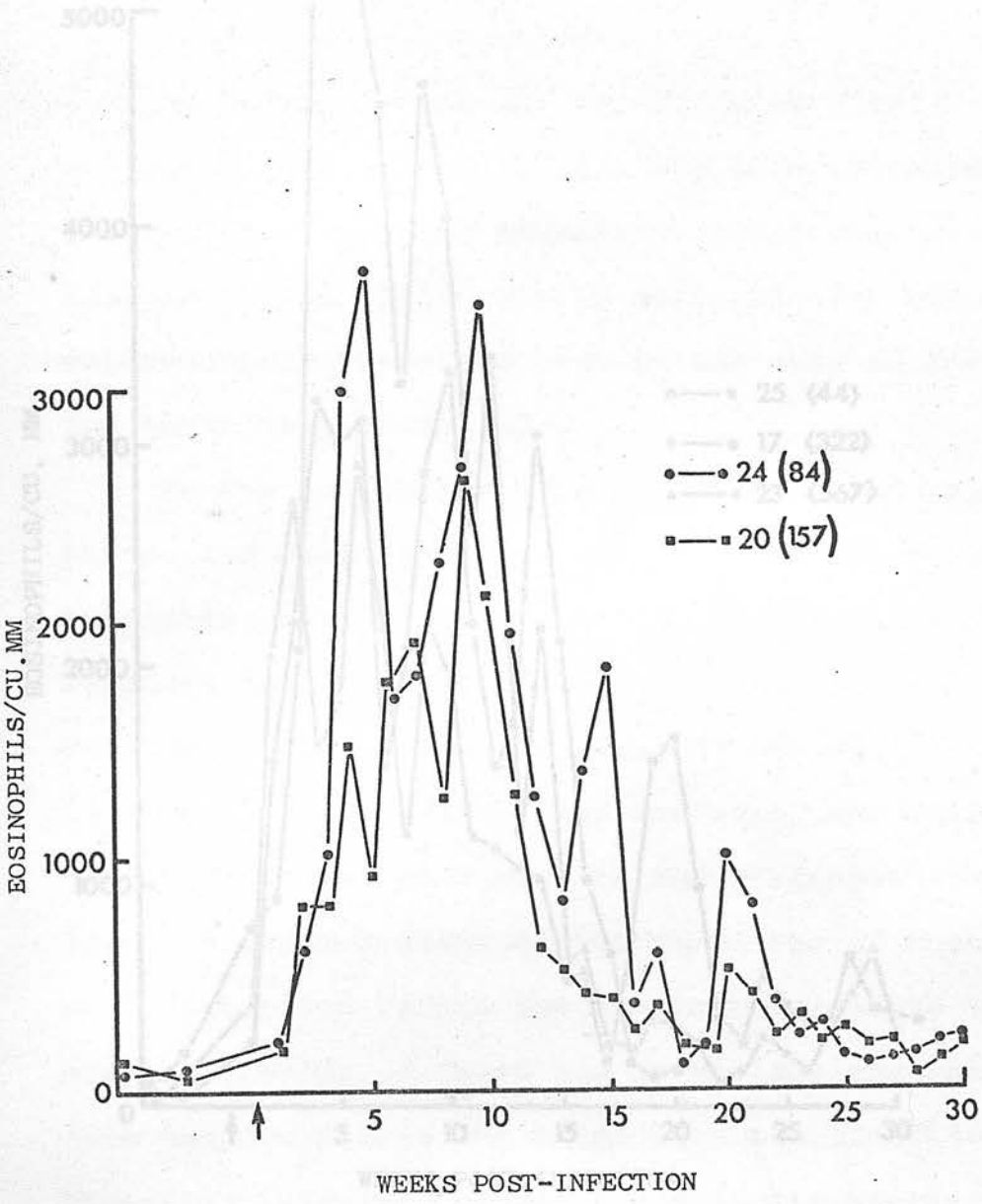
4. Haematological data

(a) Studies using radio-active isotopes: The results of these are given in Sewell, Hammond and Dinning (1968), a copy of which is attached to this dissertation.

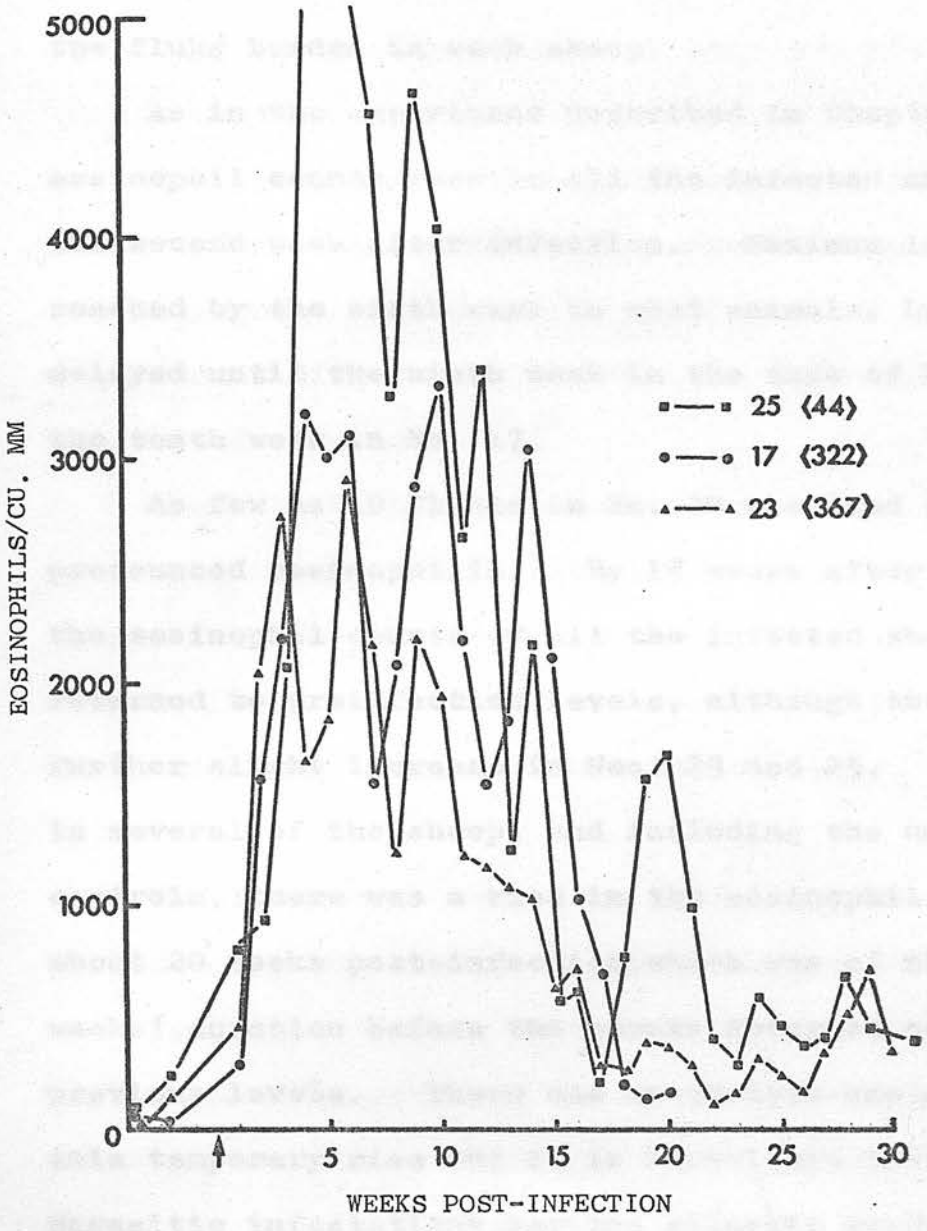
(b) The leucocyte series: It has been seen in Chapter 9 that the leucocytosis, which developed in most of the sheep, could be accounted for largely by the increase in the number of eosinophils. Accordingly the eosinophilia was studied in detail in sheep with different fluke burdens and the results are shown in Appendix Table 10.B and Graphs 10.3, 10.4 and 10.5. The figures in parenthesis on the graphs show



GRAPH 10.3 EOSINOPHIL COUNTS



GRAPH 10.4 EOSINOPHIL COUNTS



GRAPH 10.5 EOSINOPHIL COUNTS

the fluke burden in each sheep.

As in the experiment described in Chapter 9 the eosinophil counts rose in all the infected animals by the second week after infection. Maximum levels were reached by the sixth week in most animals, but were delayed until the ninth week in the case of No. 20 and the tenth week in No. 17.

As few as 10 flukes in No. 30 resulted in a pronounced eosinophilia. By 18 weeks after infection the eosinophil counts of all the infected sheep had returned to preinfection levels, although there was a further slight increase in Nos. 23 and 25. However in several of the sheep, and including the uninfected controls, there was a rise in the eosinophil counts at about 20 weeks post-infection which was of about 2 weeks' duration before the counts returned to their previous levels. There was no obvious explanation for this temporary rise but it is known that besides parasitic infestations various allergic states may cause eosinophilia (Archer, 1963). It is possible that such an allergic state may have been responsible in this case.

It is apparent that eosinophilia of the peripheral blood is not an index of the intensity of the infection as there was no consistent correlation between the eosinophil count and the fluke burden. Thus sheep 25 with 44 flukes had very much higher eosinophil counts than any other of the infected sheep,

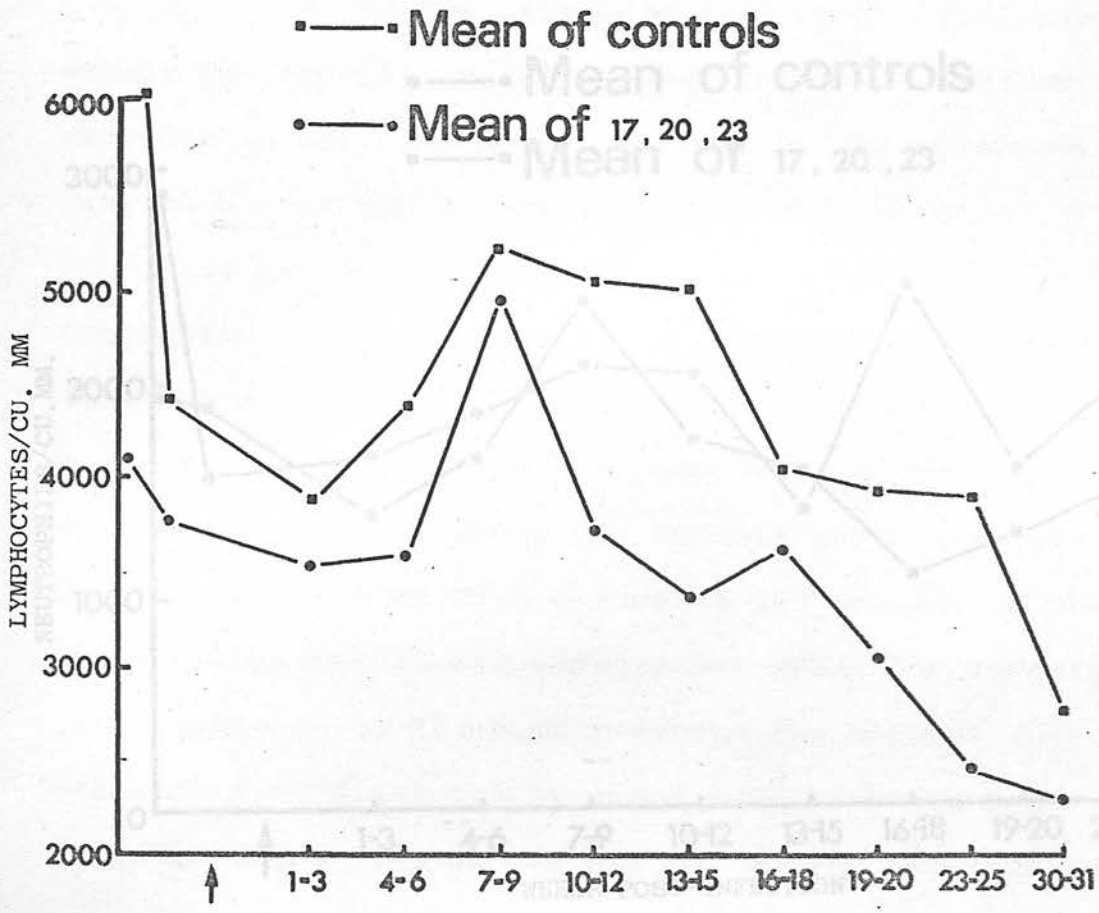
including those with burdens of over 300 flukes (Graph 10.5).

The total and differential leucocyte counts are shown in Appendix Table 10.B. However, apart from confirming the eosinophilia, there were no other significant differences between the infected and the uninfected animals.

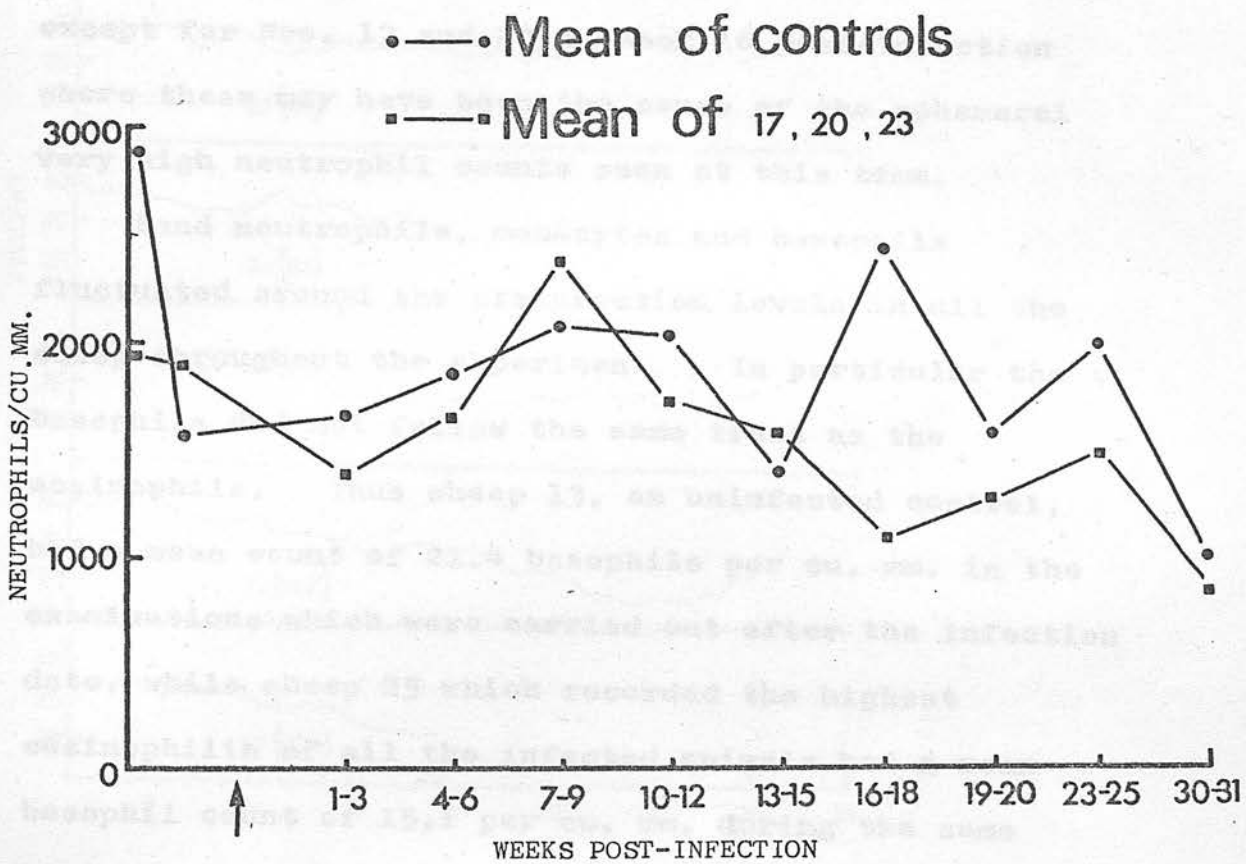
Graph 10.6 shows lymphocyte counts and Graph 10.7 neutrophil counts. In both the graphs the mean of the counts for the uninfected controls (Nos. 13 and 14) are compared with the mean of the counts for the three sheep with the heaviest fluke burdens (Nos. 17, 20 and 23).

The lymphocyte counts in individual sheep varied considerably but throughout the experimental period the mean lymphocyte counts in the uninfected sheep were higher than the means of the three infected sheep, although both these means tended to parallel each other. There was a slight lymphocytosis at 7-9 weeks after infection, but this also occurred and persisted longer in the uninfected sheep.

The neutrophil counts tended to increase from the levels at the time of infection in both infected and uninfected animals, reaching a peak at 7-9 weeks post-infection in the case of the infected group, which was followed by a gradual fall. The control group however did not reach such a high level at 7-9 weeks post-infection but later reached a peak level higher



GRAPH 10.6 LYMPHOCYTE COUNTS



GRAPH 10.7 NEUTROPHIL COUNTS

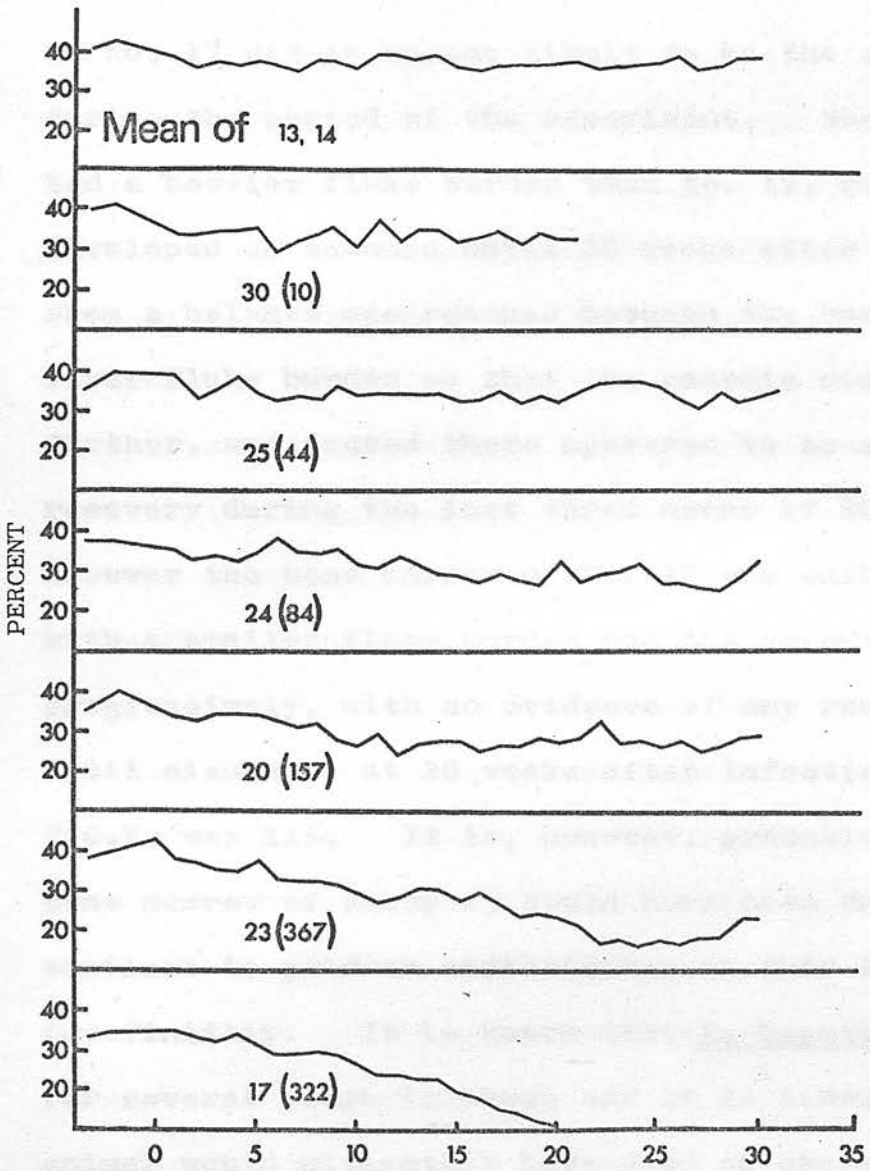
than that in the infected group.

No toxic granulation, or other indications of toxaemia, were seen in the neutrophils of the infected sheep. The mild respiratory infections appeared to have no effect on the leucocyte pictures of the sheep, except for Nos. 13 and 25 on week 16 post-infection where these may have been the cause of the ephemeral very high neutrophil counts seen at this time.

Band neutrophils, monocytes and basophils fluctuated around the preinfection levels in all the sheep throughout the experiment. In particular the basophils did not follow the same trend as the eosinophils. Thus sheep 13, an uninfected control, had a mean count of 21.4 basophils per cu. mm. in the examinations which were carried out after the infection date, while sheep 25 which recorded the highest eosinophilia of all the infected animals had a mean basophil count of 15.1 per cu. mm. during the same period.

(c) <u>The erythrocyte series</u>: The results are given in Appendix Table 10.C and Graph 10.8. In Graph 10.8 the figures in parenthesis show the fluke burden in each sheep.

Only sheep 17, 20, 23 and 24 showed a drop in P.C.V. In the case of Nos. 17, 20 and 23 this fall commenced about 5 weeks post-infection, but was delayed in No. 24 until 9 weeks after infection. In all four animals the onset of the anaemia was gradual, but only



GRAPH 10.8 PACKED CELL VOLUMES

in No. 17 did it appear likely to be the cause of death during the period of the experiment. Sheep 23, which had a heavier fluke burden than No. 17, progressively developed an anaemia until 22 weeks after infection when a balance was reached between the host and its liver fluke burden so that the anaemia did not develop further, and indeed there appeared to be some degree of recovery during the last three weeks of the experiment. However the bone marrow of No. 17 was unable to cope with a smaller fluke burden and the anaemia developed progressively, with no evidence of any remission, until slaughter at 20 weeks after infection when the P.C.V. was 11%. It is, however, probable that the bone marrow of sheep 23 would have been unable to continue to produce erythrocytes at this level indefinitely. It is known that F. hepatica may live for several years in sheep and it is likely that this animal would ultimately have died of anaemia.

A similar situation to that in sheep 23 occurred in No. 20 although the degree of anaemia was much less. Here again there was some remission of the anaemia at the end of the period of the experiment. The anaemia which developed in sheep 24 was only slightly less than that in No. 20 and again there was some remission of symptoms at the end of the experimental period.

Abnormalities in the erythrocyte picture were seen only in sheep Nos. 23, 20, 17 and 24, but not until 13 weeks after infection. These abnormalities were most

marked in sheep No. 23 where the incidence of polychromasia, punctate basophilia and anisocytosis increased until the end of the experiment. Poikilocytosis was not seen until 23 weeks after infection but was then also progressive. A few late normoblasts were also found in blood smears from this sheep taken 23, 27 and 30 weeks post-infection. Plate I (Sewell, Hammond and Dinning, 1968, attached) shows a stained blood smear from sheep No. 23, made 30 weeks after infection.

The same general picture was seen in sheep No. 17, but these changes were not so marked in the case of sheep No. 20 and furthermore the erythrocyte picture had returned to normal 30 weeks after infection. Abnormalities in the red cell picture of sheep No. 24 were confined to the appearance of punctate basophilia 18 weeks after infection.

The haemoglobin concentrations and red blood cell counts both showed similar trends to the P.C.V. in all the animals.

Values for the mean corpuscular volume (M.C.V.) showed no significant changes as a result of the infection, and those for the mean corpuscular haemoglobin (M.C.H.) were also little changed. In some of the infected sheep and in one of the controls the mean corpuscular haemoglobin concentrations (M.C.H.C.) fell slightly below 30% thus indicating a hypochromia, according to Schalm (1965). However

these low values were marginal, irregular, not progressive and not related to the fluke burdens.

(d) Bone marrow studies: The erythropoietic activity of one femur from each sheep is shown in Table 10.3. There is a clear correlation between the increase in erythropoietic activity and the fluke burden. This difference was most noticeable in the femurs which showed little erythropoietic activity in the normal sheep, but considerable activity in the infected animals.

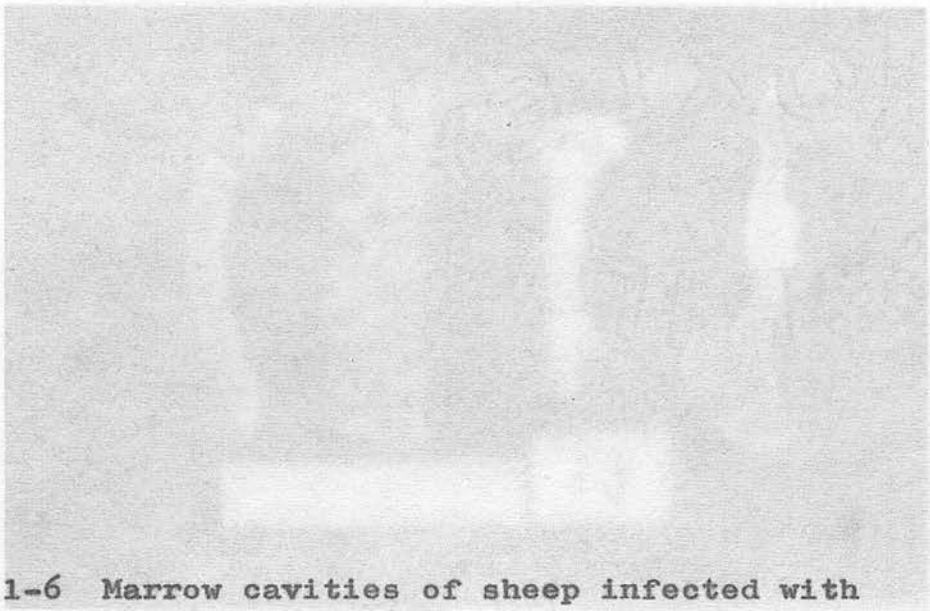
Table 10.3

Erythropoietic activity of femur (%)

Sheep No.	13	14	30	25	24	20	17	23
Percent cross-sectional area of erythropoietic marrow to cross-sectional area of femur	2.6	6.8	9.7	11.9	13.5	17.2	24.7	26.8
No. of flukes found post-mortem	0	0	10	44	84	157	322	367

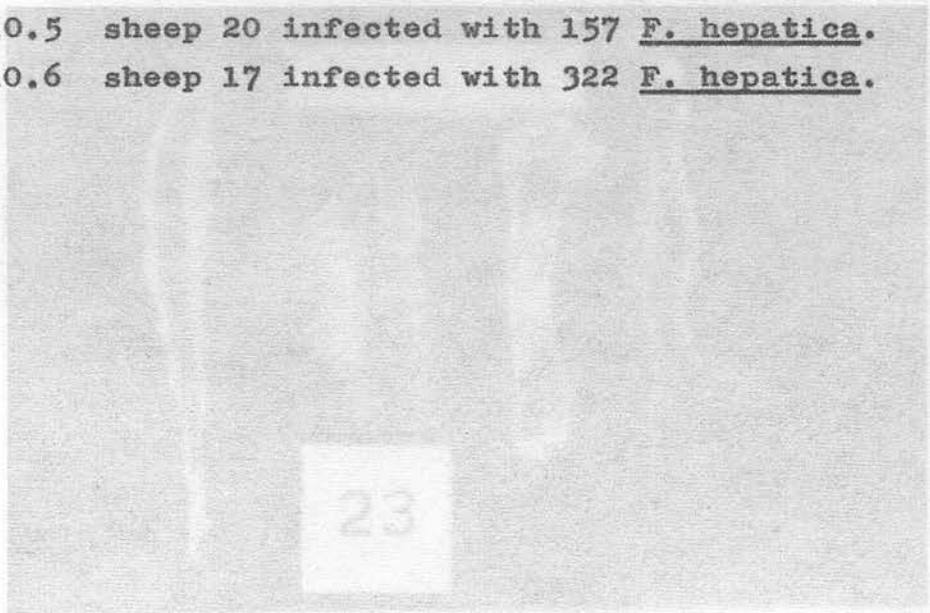
The displayed marrow cavities of the sternum, lumbar vertebrae, one femur, and one ileum of some of the sheep are shown in Plate 10.1-6.

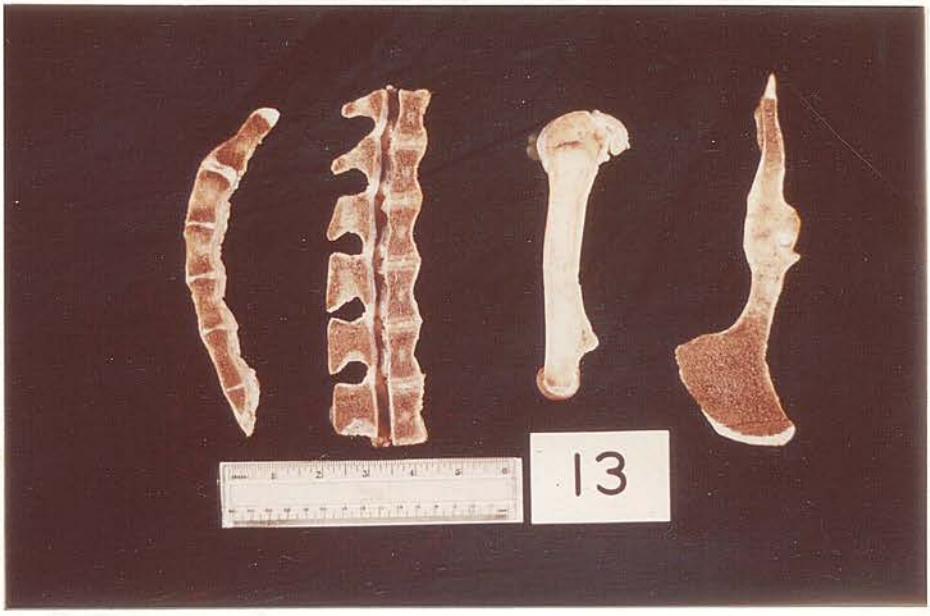
It was necessary to clean these preparations by removing congealed fat and clotted blood and during this process some of the erythropoietic marrow tissue may have been lost. This may account for some lack of



Plates 10.1-6 Marrow cavities of sheep infected with Fasciola hepatica.

10.1 sheep 13 uninfected control
10.2 sheep 23 infected with 367 F. hepatica.
10.3 sheep 14 uninfected control.
10.4 sheep 25 infected with 44 F. hepatica.
10.5 sheep 20 infected with 157 F. hepatica.
10.6 sheep 17 infected with 322 F. hepatica.

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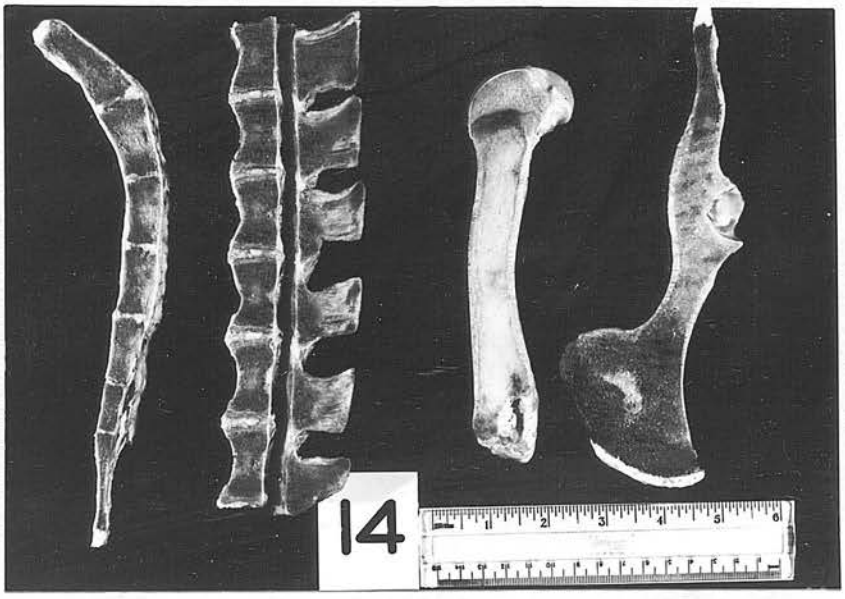
23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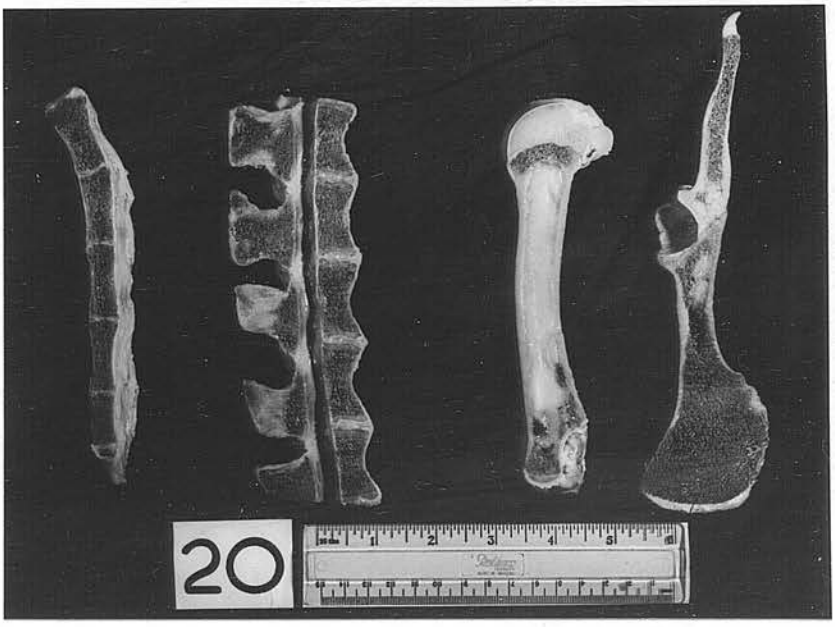
25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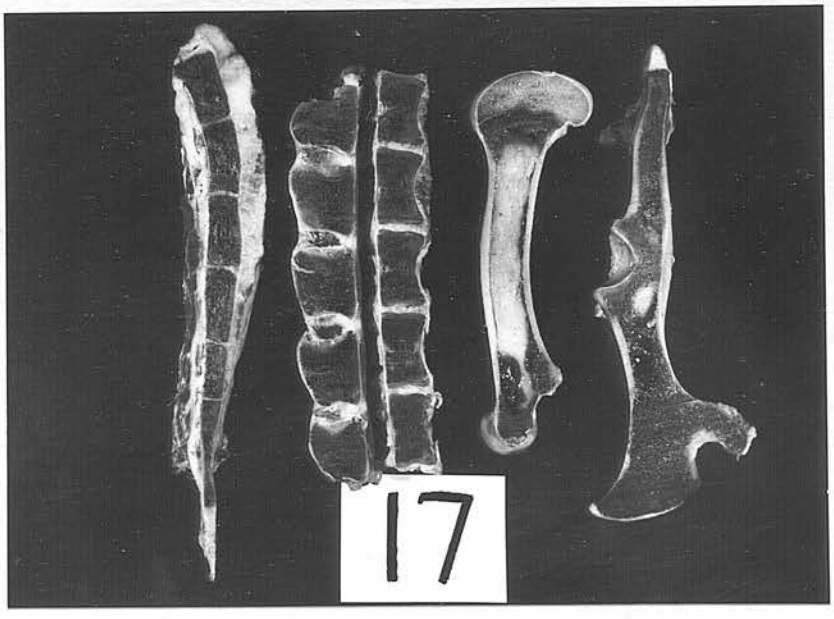

17

colour.

Bone marrow is mixed with adipose tissue which increases with the age of the animal and it is therefore important that comparisons are made between animals of the same age. In this experiment sheep 17 and 30 were 16 months old and all the other sheep 19 months old at slaughter.

The correlation between the marrow activity and the liver fluke burden can be clearly seen in the plates and especially in the femur of each animal.

As the fluke burden increases, the areas of pale fatty inactive marrow, which are prominent in the bodies of the lumbar vertebrae and sternebrae of the uninfected controls, Nos. 13 and 14, and in No. 25, disappear and are replaced by active tissue. Thus, these pale fatty areas are not seen in the lumbar vertebrae of No. 24, but are faintly visible in some of the sternebrae. However they are not seen at all in Nos. 20, 17 and 23, the three most heavily infected sheep. Fatty areas are seen in the ilea of the more heavily infected sheep, Nos. 20 and 17, although much reduced in size compared to the sheep with lighter infection. However they are not visible in No. 23 which had the heaviest fluke burden of all. Areas of very cellular, dark red gelatinous erythropoietic material are only seen in the femur and ileum and only in the three most heavily infected sheep. These areas are not extensive in No. 20 but are much more prominent

in Nos. 23 and 17.

(e) Vitamin B_{12} and plasma folate concentrations: The results of the vitamin B_{12} assays are shown in Table 10.4. It is apparent that although the concentration of vitamin B_{12} fell, as compared with the preinfection levels in the most heavily infected sheep, similar or larger falls occurred in sheep 30 and 25 with fluke burdens of 10 and 44 and in the uninfected control, No. 14. In contrast, No. 24 with 84 flukes showed a rise. It would therefore seem unlikely that a vitamin B_{12} deficiency is consistently associated with chronic infection of this length.

Table 10.4

Serum vitamin B_{12} concentrations (μμg./ml.)

Sheep No.	Preinfection	Weeks post-infection	
		19/20	22
13	498	256	426
23	641	-	343
25	918	-	382
14	669	354	439
30	624	249	-
20	623	-	608
17	341	221	-
24	277	-	491

The results of the plasma folate assays are shown

in Table 10.5.

Table 10.5

Plasma folate concentrations (m.µg./ml.)

Sheep No.	Preinfection	Weeks post-infection 19-22
13	1.50	1.45
23	1.90	1.30
25	1.95	1.80
14	1.15	1.50
30	1.75	1.65
20	1.45	1.60
17	1.45	1.85
24	1.70	2.30

All the concentrations are given as the means of two assays on the same or on different plasma samples.

Again it would seem that, as there is no consistent change in the plasma folate concentrations consequent upon infection, it is unlikely that a folic acid deficiency is associated with F. hepatica infections of this type.

Discussion and conclusions

No evidence was found that the anaemia of chronic fascioliasis results from a dyshaemopoiea in infections of the length described, although this may be so in more prolonged infections. It is concluded

from these studies that the anaemia of chronic fascioliasis is primarily caused by loss of blood into the bile ducts.

Furthermore no clear-cut results were obtained from the studies on vitamin B_{12} and plasma folate concentrations. It is considered unlikely that these are important factors in the aetiology of the anaemia of chronic fascioliasis. These studies were not repeated in subsequent infections as other workers failed to influence the course of the anaemia with large doses of vitamin B_{12} (Boray, 1967; Sinclair, 1967).

Great individual variation was shown from animal to animal in the response to infection with F. hepatica. Although sheep Nos. 17 and 23 had similar fluke burdens at post-mortem examination the pathogenic effects of these infections were very different in these two hosts. While No. 17 was slaughtered when moribund 20 weeks after infection, No. 23 was maintaining a balance with the parasites when slaughtered 30 weeks post-infection.

Sheep 17 weighed 12 lbs. less than No. 23 at the time of infection, although the same age, and it is likely that it was a less vigorous animal less able to resist the effects of the infection. However the difference in pathogenicity was so marked that there may have been an inherent genetical difference as well. As the metacercariae which were used to infect these

sheep were from the same batch, and were treated in the same way, the pathogenicity of the two infections was not affected by environmental factors such as described by Davtyan (1956) and by Boray (1963).

Furthermore the flukes reached a larger size (which may account for their greater pathogenicity) in a shorter time and also laid more eggs, in No. 17 than in No. 23, and this may also indicate a lack of resistance by the host.

The finding that there was some remission of the anaemia in some of the infected animals towards the end of the experimental period is not easily explained. It is unlikely to be because many flukes were dying, only 7 months after infection, as it is known that F. hepatica lives for some years in sheep (Durbin, 1952). Further, the management and feeding of these animals was not changed during the experiment.

CHAPTER 11

Chronic Fascioliasis in Sheep (Fasciola gigantica)

Introduction

In contrast to the numerous studies with Fasciola hepatica, very little work has been reported on chronic fascioliasis in sheep caused by F. gigantica. However, it is generally considered that F. gigantica is much more pathogenic than F. hepatica for sheep, and Davtyan (1953) found that as few as 31 and 34 of these liver flukes resulted in the death of sheep in a group with live-weights ranging from 32.3 to 49.2 kg. Further, Condy (1962) reported that F. gigantica is only rarely seen in sheep livers at Rhodesian abattoirs, considering that the condition is invariably acute in field outbreaks.

The present work, which was carried out in 1969, was designed to study chronic F. gigantica infection in sheep, with special emphasis on the economic effects of such infections.

Experimental design

(a) Animals

Twenty 9 month old Merino x Corriedale wethers were obtained from a farm with no recent history of fascioliasis. The faeces of all animals were checked and found to be negative for Fasciola eggs, on several occasions before the start of the experiment, which

began 5½ months after the latest time at which it would have been possible for them to have acquired a natural infection. This is well beyond the prepatent period of this liver fluke in sheep. The management and feeding of these sheep have already been discussed.

(b) Treatments

The sheep were divided into two groups of 10 animals so that the mean live-weight for both groups was the same. The group to be infected was then selected at random and each sheep in it was given 60 metacercariae. The other group was used as uninfected controls. At the time of infection the metacercariae were 9 days old.

(c) Observations

All sheep were weighed weekly throughout the experiment. Packed cell volumes (P.C.V.s) were determined preinfection and at intervals. Fasciola egg counts were carried out monthly and nematode egg counts were also carried out on some sheep at irregular intervals.

Twenty-nine weeks after infection all the sheep were slaughtered and their liver fluke burdens recovered. A detailed carcass and wool analysis was carried out on each sheep.

Results

1. Clinical data

No clinical symptoms of fascioliasis were seen at

any time during the experiment, and it was not possible to distinguish the infected sheep from the controls.

Several sheep had nasal discharges especially during the rainy season. One of these, X211, was treated with 'Sulphamezathine' (sulphadimidine, I.C.I.) but the infection resolved spontaneously in the other sheep.

The changes in live-weight are shown in Appendix Table 11.A, in Table 11.1 and in Graph 11.1.

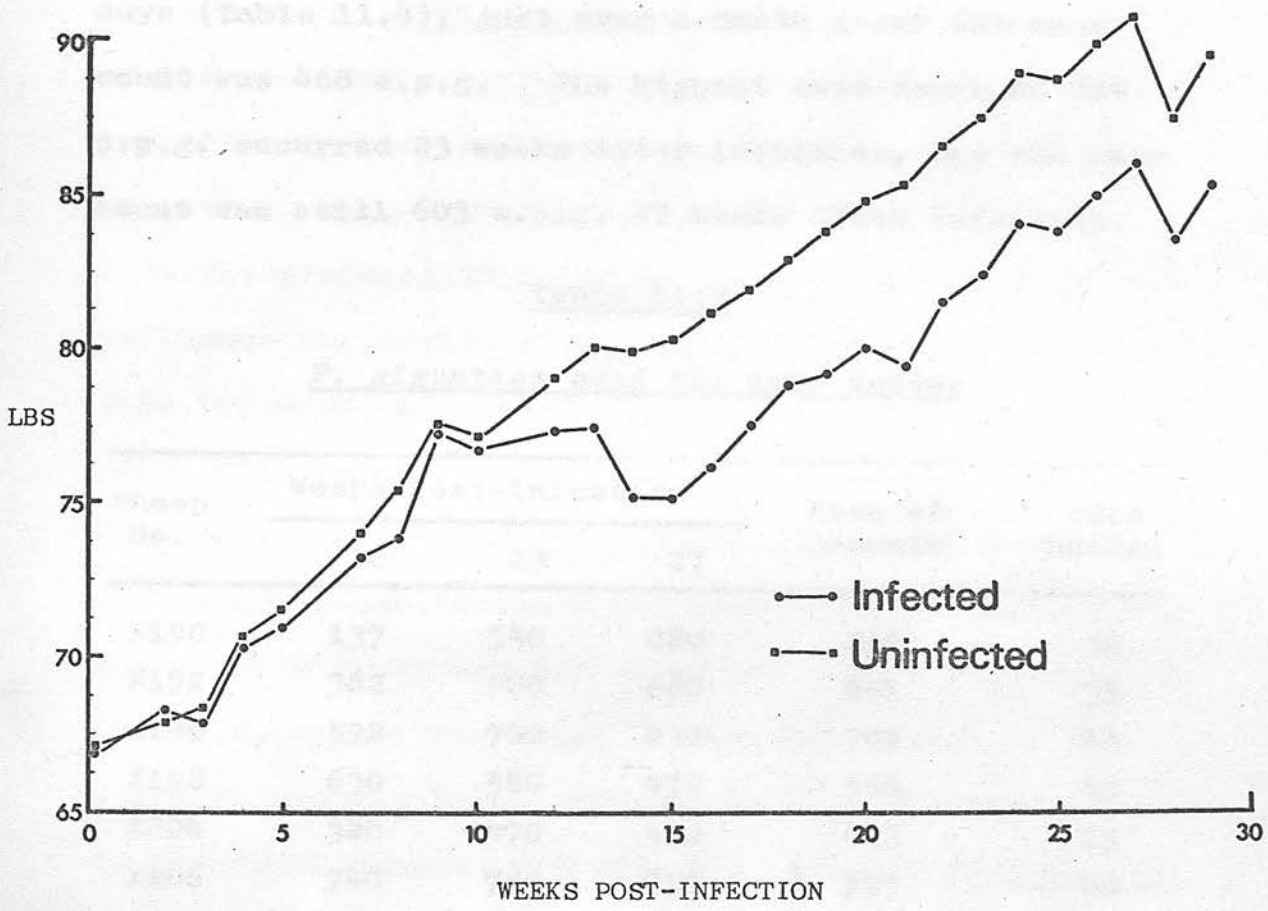
Table 11.1

Mean live-weight changes

Live-weights (lbs.)	Infected	Control	t	significance
Initial unstarved weight	66.7	66.7	-	-
Gains 0-16 weeks post-infection	9.4	14.4	4.39	< 0.001
Gains 16-29 weeks post-infection	9.1	8.8	0.29	N.S.
Gains 0-29 weeks post-infection	18.5	23.2	2.61	< 0.02

N.S. = not significant

There was virtually no difference between the groups until 10 weeks post-infection, after which the infected group received a severe check until 15 weeks post-infection, when live-weight gains continued once more at about the same rate as in the uninfected group. The period 10-15 weeks after infection is from when the flukes are probably beginning to enter the bile ducts until shortly after the infection becomes patent.



GRAPH 11.1 LIVE-WEIGHTS (MEANS OF GROUPS)

2. Parasitological data

The results for the counts of F. gigantica eggs in the faeces are given in Table 11.2. The prepatent period ranged from 90-105 days with a mean of 95.1 days (Table 11.4), just over a month later the mean count was 468 e.p.g. The highest mean count of 704 e.p.g. occurred 23 weeks after infection, and the mean count was still 603 e.p.g. 27 weeks after infection.

Table 11.2

F. gigantica eggs per gram faeces

Sheep No.	Weeks post-infection			Mean of counts	Fluke burden
	18	23	27		
X190	137	340	280	252	38
X192	362	900	660	641	37
X196	592	700	830	707	44
X198	630	580	470	560	42
X204	320	470	490	423	25
X206	740	780	650	723	40
X211	310	420	510	413	40
X214	550	800	710	687	24
X215	384	1050	650	695	48
X217	660	1000	780	813	35

As in the case of F. hepatica infections in sheep there were considerable differences in egg production in animals with approximately the same fluke burden (Table 11.2).

The autopsy data from the sheep are shown in Table 11.4. The mean percentage recovery of flukes

was 62.2±12.79. Sheep X211 had 7 flukes which had died and had started to degenerate posteriorly, while X190 and X217 each had 2 such degenerating flukes. This was an unexpected finding as it was thought that the longevity of F. gigantica in sheep would be similar to that of F. hepatica, at this low level of infection. Only one fluke was found in the small intestine, this being in X190.

The presence of Stilesia hepatica appeared to influence the number of F. gigantica which developed. This tapeworm was present in the livers of X204, X206, X214, X215 and X217 which had a total fluke population of 172, with a mean of 34.4±10.16 flukes per sheep. It was not found in the other five infected sheep which had a total fluke population of 201 with a mean of 40.2±2.86 flukes per sheep. However, little significance should be attached to this finding because of the small sample involved, and moreover S. hepatica, although present in the two sheep with the smallest number of flukes, was also found in that with the largest number. Again, the number of Stilesia found in the livers was not recorded. Five of the control sheep were also infected with S. hepatica.

Egg production was not adversely affected by the presence of S. hepatica, for the 34.4 F. gigantica per sheep with the tapeworm present produced a mean of 668 e.p.g. for the three counts (Table 11.2), while the 40.2 flukes per sheep with no tapeworms present

produced a mean of only 515 e.p.g. for the same counts.

Similar interference between Fasciola and other liver parasites have been discussed by Froyd (1960), who found in a survey in Kenya that the presence of hydatid cysts (Echinococcus granulosus) in cattle livers appeared to cause a reduction of over 20% in the expected infection rate with F. gigantica. However, Lang (1967) found no such association between infections with Hymenolepis microstoma and F. hepatica in mice.

Nematode egg counts were carried out periodically to find when treatment with thiabendazole (Merck, Sharpe and Dohme) was indicated. Some sheep regularly had a much higher egg count than others, and the infected group had higher counts than the non-infected group except at the terminal counts when they were almost equal. Again, the sample was small, five sheep from each group, and the results were biased because two of the non-infected sheep usually had negative counts while X217, an infected sheep, had uniformly high counts.

3. Haematological data

The results of the P.C.V. estimations are shown in Table 11.3. By comparison with the controls the infected group had some reduction in mean values which was greatest at the 15 weeks post-infection determinations. The fall in the mean P.C.V. of the infected group was not progressive however and values

Table 11.3

Packed cell volume (%)

Infected group

Sheep No.		X190	X192	X196	X198	X204	X206	X211	X214	X215	X217	Mean
Preinfection		30½	33½	34½	32½	30½	34½	30½	33½	31½	38	33.0
Weeks post-infection	15	25	25	26½	28	25	28	28½	29½	25½	32½	27.4
	28	27	31½	29½	31	32½	33½	30½	32	29½	34	31.1
	29	32	33½	30	31	31½	35½	32½	33½	32½	35½	32.8

Uninfected group

Sheep No.		X189	X195	X197	X199	X201	X203	X207	X208	X209	X212	Mean
Preinfection		33½	33½	33	31	32	34½	31½	32½	34½	32	32.8
Weeks post-infection	15	31½	33	31	35	31	33	31	31½	34½	34½	32.6
	28	31½	34	34½	35½	31	34	32	34	33	35	33.4
	29	33½	37½	37½	37½	34½	39	36	36½	36½	38½	36.7

rose from this level to reach those found preinfection, at 29 weeks. Nevertheless they were still below those of the control group.

4. Post-mortem examinations

Some results of the post-mortem examinations are recorded in Table 11.4. No evidence of liver fluke infection was found in any of the control group. The only lesions found in the infected sheep were those in the liver and as they were indistinguishable from chronic fascioliasis caused by F. hepatica they will not be described in detail. The hepatic bile ducts were very enlarged and the walls were thickened, up to 2 mm. thick in some cases. Calcification was not detected, but the livers of two sheep, X196 and X204, each had an inspissated abscess of about 2 cm. diameter. The livers of the infected sheep were enlarged, with a mean weight of 462 g.±37.54, compared to the controls with a mean weight of 390 g.±49.94; the mean weight of the hepatic lymph nodes of the infected sheep was four times that of the control sheep - 6.2 g. compared with 1.5 g.

5. Carcass analyses and other data

The carcass analyses were carried out at E.A.A.F.R.O. by Mr. H. P. Ledger and his staff. The carcass evaluation procedures adopted were based largely on the system recommended by the Agricultural Research Council, London (1965).

The results of the carcass analyses are given in

Table 11.4

Some experimental details

Sheep No.	X190	X192	X196	X198	X204	X206	X211	X214	X215	X217	Mean
Infective dose of metacercariae	60	60	60	60	60	60	60	60	60	60	60
No. of F. gigantica recovered	38	37	44	42	25	40	40	24	48	35	37.3
% recovery	63.3	61.7	73.3	70.0	41.7	66.7	66.7	40.0	80.0	58.3	62.2
Prepatent period (days)	95	98	94	90	95	94	95	105	95	90	95.1
Weight of liver (g.)	455	466	511	508	508	434	467	402	440	429	462
Weight of hepatic lymph nodes (g.)	-	5.5	8.8	6.0	5.5	7.0	4.3	6.0	8.5	5.0	6.2
Weight of empty gall bladder (g.)	3.0	2.5	4.5	2.8	3.0	3.5	3.8	3.0	3.5	3.0	3.3
Vol. of bile (ml.)	9.0	10.8	18.2	9.8	-	17.6	18.2	8.8	8.4	5.0	11.8

Sheep No.	X189	X195	X197	X199	X201	X203	X207	X208	X209	X212	Mean
Infective dose of metacercariae	-	-	-	-	-	-	-	-	-	-	-
Weight of liver (g.)	466	339	430	395	375	366	344	452	320	418	390
Weight of hepatic lymph nodes (g.)	2.0	1.0	1.8	1.3	2.0	1.0	1.8	1.8	1.3	1.0	1.5
Weight of empty gall bladder (g.)	2.5	2.5	3.0	2.5	2.8	2.8	2.0	3.8	2.0	2.0	2.6
Vol. of bile (ml.)	9.0	8.0	15.2	7.0	4.8	7.4	10.4	16.4	9.4	3.2	8.1

full in Appendix Table 11.B, and the mean figures are compared in Table 11.5.

Table 11.5

Analysis of carcass data

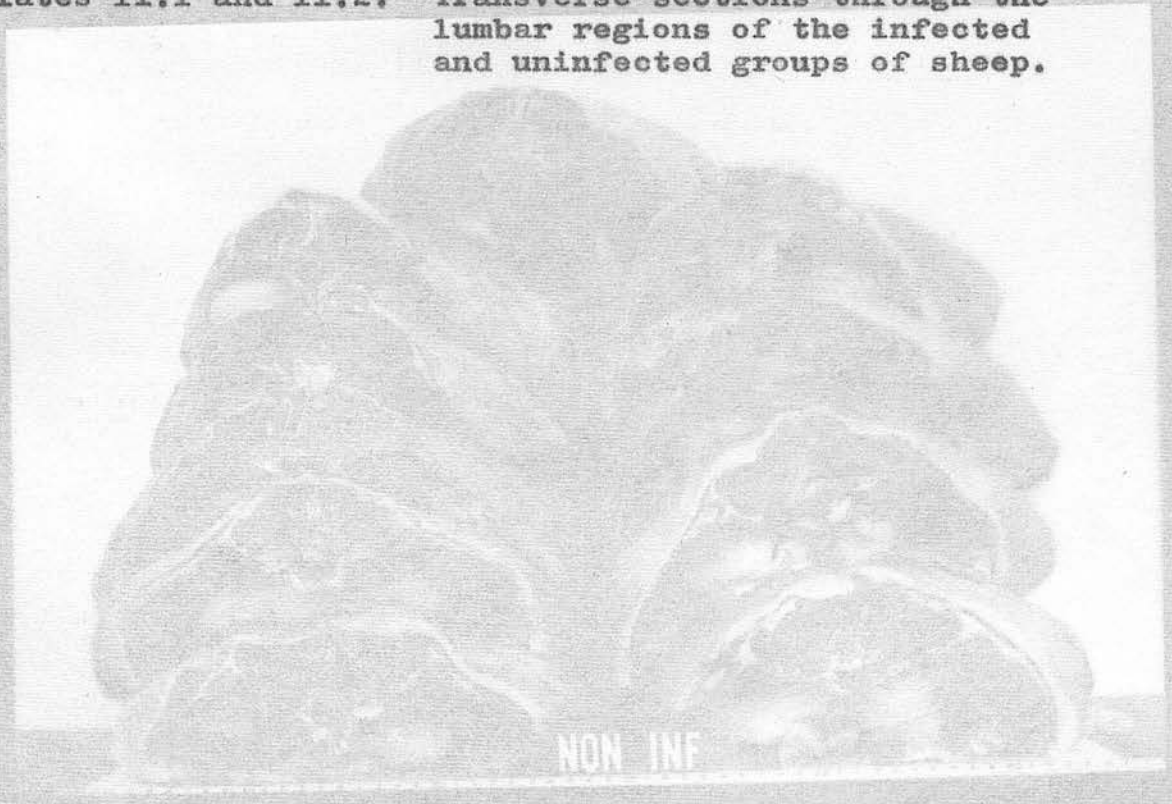
	Mean values		t	Sig.
	Infected	Control		
Live-weight (24 hour fasting), kg	35.68	37.82	1.64	N.S.
Warm dressed carcass, kg	14.15	15.17	1.43	N.S.
Cold dressed carcass (24 hour), kg	13.90	14.90	1.42	N.S.
Weight of spleen	0.0930	0.0925	0.055	N.S.
Weight of lean	9.03	9.20	0.60	N.S.
Weight of fat	2.24	3.05	2.08	<0.10
Weight of bone	2.54	2.58	0.37	N.S.
Weight of kidneys	0.078	0.077	0.13	N.S.
Lean/bone ratio	3.55	3.57	0.24	N.S.
Killing out %	39.36	39.34	0.19	N.S.
Lean %	65.0	61.9	2.19	<0.05
Fat %	16.1	20.1	2.04	<0.10
Bone %	18.4	17.5	0.38	N.S.
Kidneys %	0.55	0.53	1.00	N.S.
Carcass rating score	2.7	3.7	2.36	<0.05

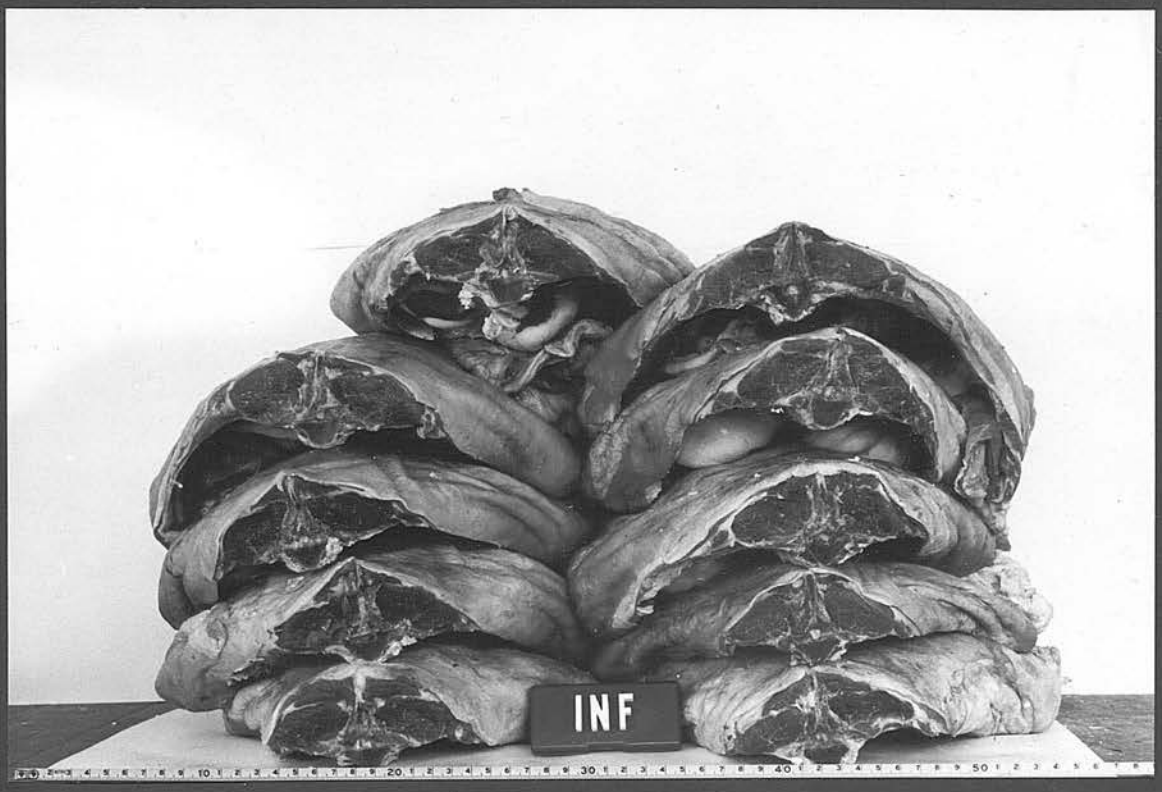
N.S. = not significant

Plates 11.1 and 11.2 show transverse sections through the lumbar regions of both groups and give a clear idea of the differing degree of finish.

The fleeces of all sheep were examined and graded by W. Schroeter, Kenya Farmers' Association (Co-operative) Wool Centre, who reported that the mean

Plates 11.1 and 11.2. Transverse sections through the lumbar regions of the infected and uninfected groups of sheep.

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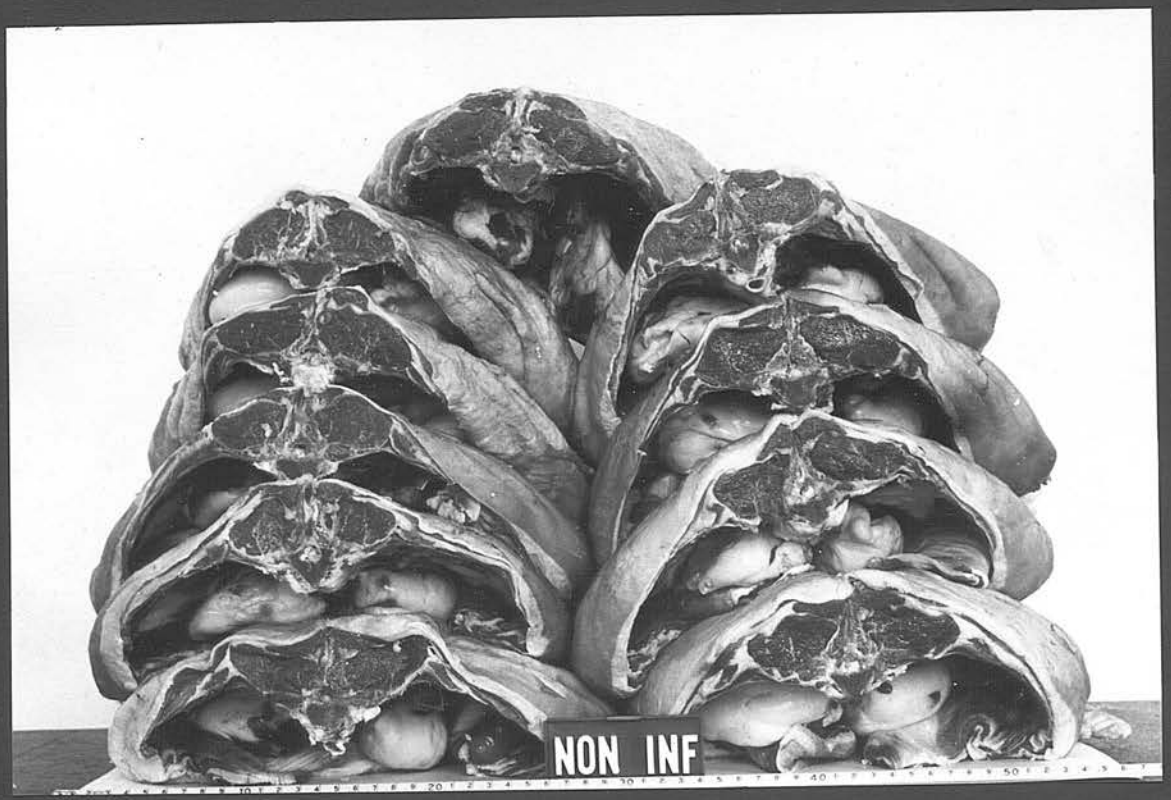

NON INF

weight of fleece-wool per infected sheep was 4.75 lb. ±0.35 compared to 5.40 lb. ±0.84 per control sheep. However, there was no difference in the mean value of fleece-wool per lb. between the two groups.

Discussion and conclusions

In studies carried out on chronic F. gigantica infections in sheep over a period of 7 months it was established that sheep can be important in the epidemiology as large numbers of eggs were passed in the faeces. The finding of dead and degenerating flukes in these 7 month old infections suggests that F. gigantica may not have such a long life span as F. hepatica in sheep.

At this level of infection the uninfected sheep gained significantly more weight than the infected sheep over the period from preinfection to 16 weeks after infection (Table 11.1). However, these differences arose entirely during the period 10 to 15 weeks post-infection when the infected group lost weight, whereas the control group maintained its previous rate of weight gain.

The control group was heavier, better finished and graded better than the infected group because of a higher proportion of fat. The infected group had less lean meat than the control group and although the percentage of lean meat was higher in the former group this was only because the amount of fat was less.

There was very little difference in the weights of bone and kidneys.

While the mean weight of fleece-wool per infected sheep was clearly less than that of the controls, there was no difference in the mean value per lb. between the two groups.

CHAPTER 12

Chronic Fascioliasis in Cattle (Fasciola gigantica): Single Infections

Introduction

There are very few reports in the literature of studies on the pathology and pathogenesis of chronic fascioliasis in cattle caused by infection with Fasciola gigantica. When the present studies were carried out during 1967 and 1968 the only published work was that of Sewell (1962, 1966) in Nigeria. At that time no experimental work had been published from East Africa, although Coyle (1956, 1958 and 1961) and Weinbren and Coyle (1960) had made some observations on naturally infected cattle in Uganda. Since then there have been a series of publications by Bitakaramire (1968, 1969) and Bitakaramire and Bwangamoi (1969) in Kenya.

An experiment was therefore designed with the aim of correlating the development of the parasite and of the lesions with the observed haematological and biochemical changes in the living animal.

Experimental design

(a) Animals

Twelve Guernsey-type castrated male calves were obtained from a farm with no recent history of fascioliasis and maintained as previously described. The calves were 3 months of age on arrival.

(b) Treatments

At 7 months of age the calves were randomly divided into 4 lots of 3 animals. One calf in each was infected with 1000 metacercariae of F. gigantica and another with 2000 metacercariae while the third was left uninfected as the control. The 4 calves which had each been infected with 1000 metacercariae were designated Group 1, and those which each received 2000 metacercariae Group 2, while the 4 uninfected controls were called Group C (Table 12.2).

The metacercariae used to infect these calves were from 2 to 13 days old.

One lot of 3 calves was slaughtered after each period of 4 weeks. The experiment therefore extended over a period of 4 months from the time of infection.

(c) Observations

Red cell and total leucocyte counts, haemoglobin estimations and packed cell volumes were determined at weekly intervals. At the same time blood smears were made and differential leucocyte counts carried out. Direct eosinophil counts were not made as Schalm (1965) had found that the indirect method was equally efficient in evaluating trends in eosinophil numbers.

Serum was collected at weekly intervals for use in serum enzyme assays, iodine flocculation tests and protein-bound hexose estimations.

Nematode and trematode egg counts were made on faeces collected at irregular intervals and the

prepatent period for F. gigantica was determined. At the post-mortem examination the livers were divided into the 3 areas (Area 1 the dorsal part, Area 2 the central and Area 3 the ventral parts, of the liver) described by Ross, Todd and Dow (1966) and the flukes from each area were recovered and counted separately. The lengths of all the undamaged flukes were measured.

Estimates were made of the number and genera of the gastro-intestinal nematodes present in each calf.

All the calves were weighed at weekly intervals.

Results

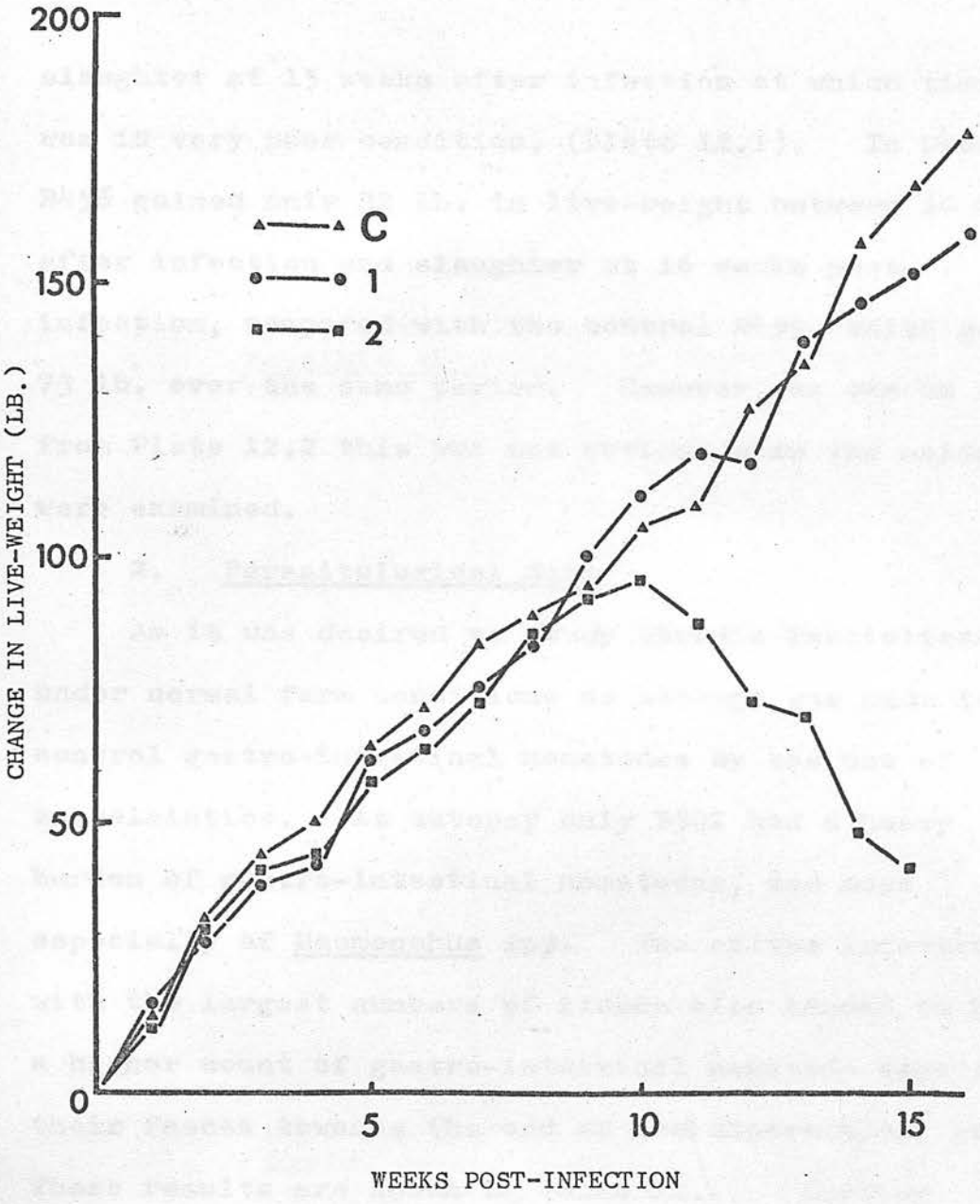
1. Clinical data

There was no apparent difference between any of the calves which had been infected with 1000 metacercariae (Group 1) and the control calves (Group C) throughout the experiment. Moreover the calves which had each been infected with 2000 metacercariae (Group 2) appeared to thrive as well as the controls until 10 weeks after infection when B502 began to lose condition slightly. By 11½ weeks after infection this animal was in noticeably poor condition and with a very reduced appetite and slight constipation. These symptoms were accompanied by a rectal temperature of 103.2°F in the morning and 105.2°F in the afternoon, and a marked leucocytosis. A course of treatment with 'Sulphamezathine' (sulphadimidine, I.C.I.) was given as it was thought that an intercurrent infection may have been responsible for these symptoms. By 13 weeks

after infection the faeces were of normal consistency although this calf continued to eat very little and was now in poor condition and was dull and lethargic. At 14 weeks after infection this calf was found to be infected with Eperythrozoon spp. and was treated with 'Spirotrypan forte' (Hoechst) at half the recommended dose. This treatment was effective in that Eperythrozoa were not seen again but the calf became progressively weaker and was slaughtered 15 weeks after infection when moribund. At this time there was also some evidence of jaundice and anaemia. The rectal temperatures during the last 2 weeks of life varied from 101.0 to 103.0°F in the morning and 100.8 to 105.7°F in the afternoon.

The other remaining calf in Group 2, B449, also began to lose its appetite 11½ weeks after infection and was then also dull and lethargic. It was slaughtered routinely 12 weeks after infection. During the last week of life the rectal morning temperature varied from 101.6 to 104.4°F.

The live-weights of the calves are shown in Appendix Table 12.A and the mean of each of the 3 groups in Graph 12.1. There was no apparent differences between the groups up to 9 weeks after infection. Thereafter the 2 remaining calves in Group 2 gained less weight than those in the other groups, and lost weight from 10 weeks after infection. B502 lost 69 lb. between 10 weeks after infection and



GRAPH 12.1 LIVE-WEIGHTS

slaughter at 15 weeks after infection at which time it was in very poor condition, (Plate 12.1). In Group 1, B456 gained only 32 lb. in live-weight between 10 weeks after infection and slaughter at 16 weeks post-infection, compared with the control B459, which gained 73 lb. over the same period. However, as can be seen from Plate 12.2 this was not obvious when the animals were examined.

2. Parasitological data

As it was desired to study chronic fascioliasis under normal farm conditions no attempt was made to control gastro-intestinal nematodes by the use of anthelmintics. At autopsy only B502 had a heavy burden of gastro-intestinal nematodes, and more especially of Haemonchus sp#. The calves infected with the largest numbers of flukes also tended to have a higher count of gastro-intestinal nematode eggs in their faeces towards the end of the experimental period. These results are shown in Table 12.1. Earlier nematode egg counts had also shown this apparent association. Thus B459, a control, had a range of 50 to 300 e.p.g. (mean 120±104) in 5 counts between 12 and 16 weeks after the infection date, while B456 of Group 1 had a range of 250 to 650 e.p.g. (mean 490±164) in similar counts. Furthermore B502 of Group 2 had a range of 700 to 3050 e.p.g. (mean 1745±1107) in 5 counts taken 12 to 15 weeks after infection. In this animal the egg counts may have been raised during this

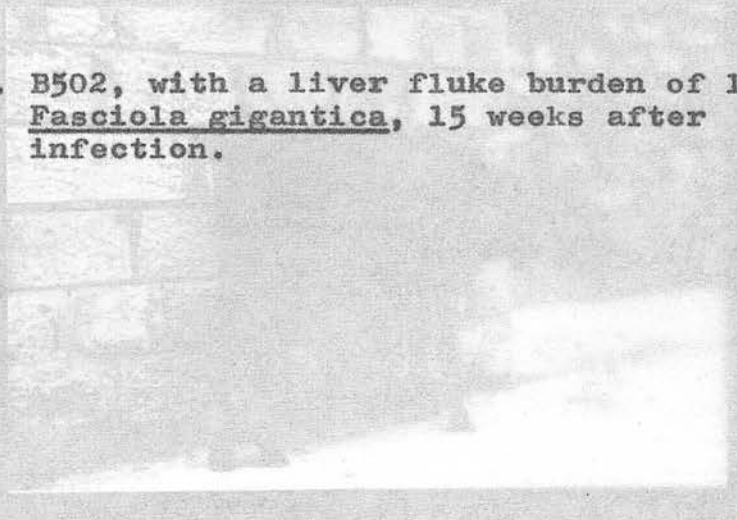


Plate 12.1. B502, with a liver fluke burden of 1387 Fasciola gigantica, 15 weeks after infection.

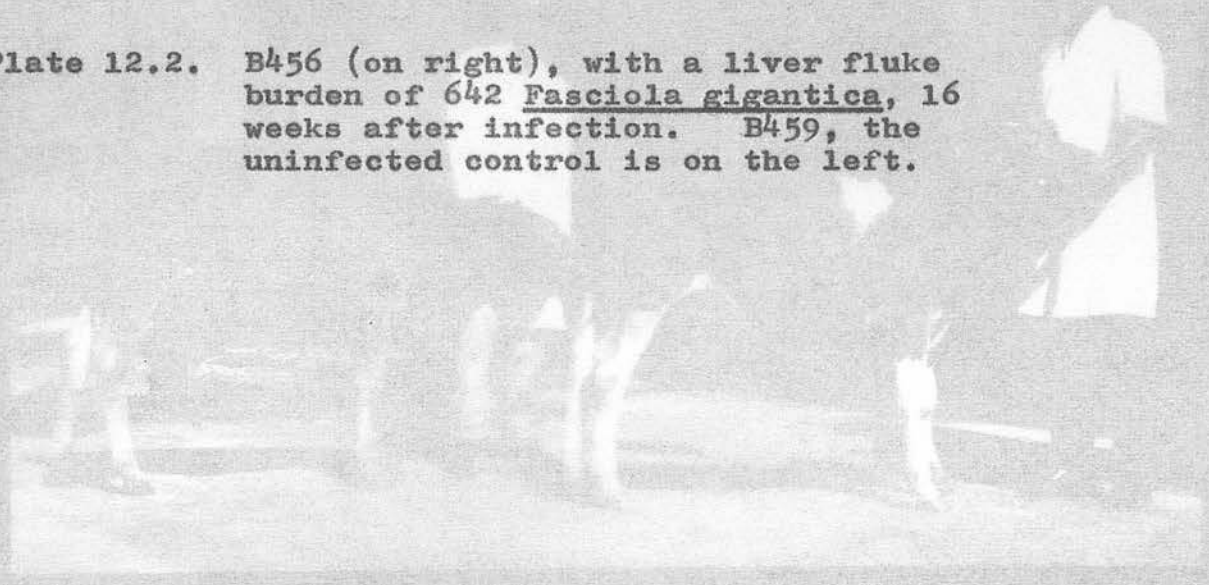


Plate 12.2. B456 (on right), with a liver fluke burden of 642 Fasciola gigantica, 16 weeks after infection. B459, the uninfected control is on the left.

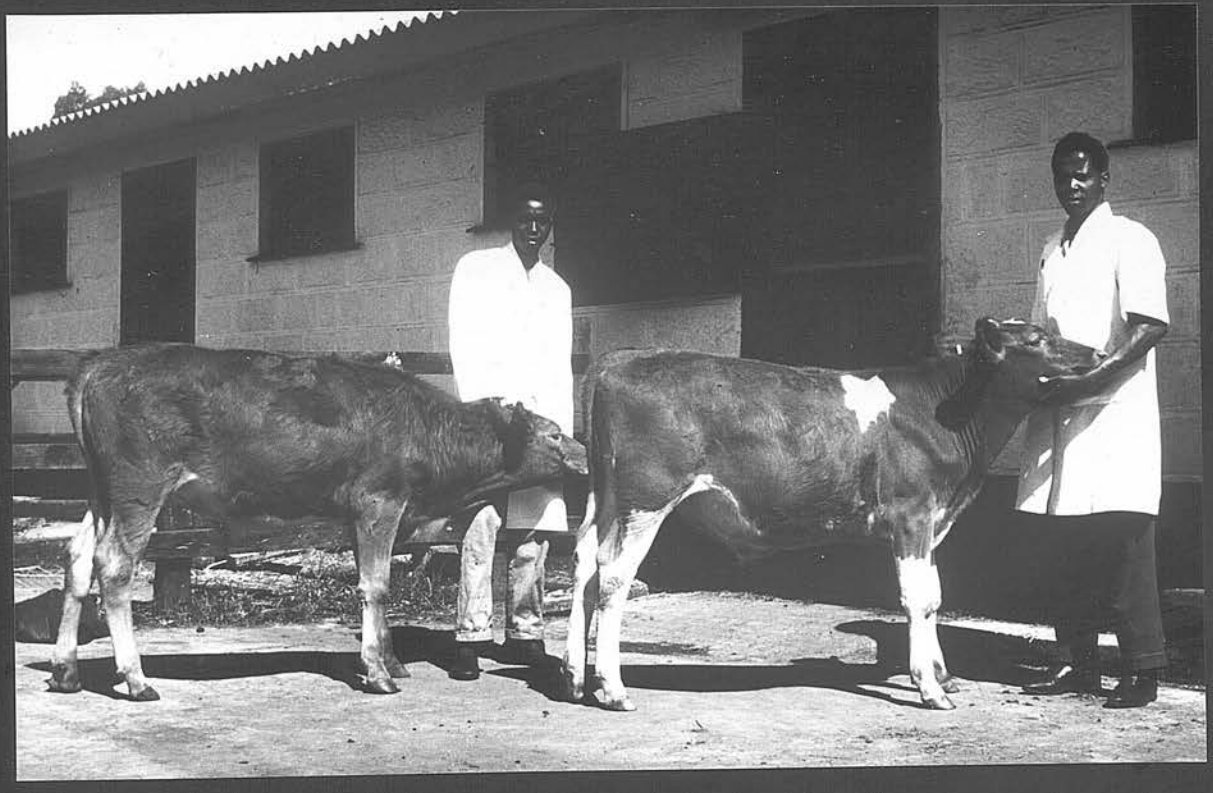


Table 12.1

Final helminth burden

	B461	B454	B460	B457	B451	B452	B453	B458	B449	B459	B456	B502
No. of *F. gigantica* recovered	-	167	596	-	590	944	-	640	1156	-	642	1387
Experimental period (weeks)	4	4	5	8	8	8	12	12	12	16	16	15
Haemonchus sp.	20	120	110*	40	30	10	150	200	160	230	140	4720
Cooperia sp.	800	510	6890	2760	3540	2010	1080	1200	3420	760+	1160+	8956
Nematodirus sp.	0	100	10	0	200	0	0	0	0	40	0	70
Trichostrongylus sp.	0	0	0	0	200	0	0	0	0	0	100	110
Bunostomum sp	10	0	0	10	0	0	0	0	0	0	10	10
Trichuris sp.	10	20	0	20	0	30	30	60	0	0	0	40
Oesophagostomum sp.	0	0	60	40	70	0	50	40	130	40	120	330
Nematode e.p.g. faeces prior to slaughter	200	350	450	100	0	200	350	350	900	300	650	1800

* Including 100 immatures
\+ Counted by naked eye only

period because it was constipated for part of the time and had a poor appetite for the whole period, but it also contained a high Haemonchus burden at autopsy.

Small numbers of coccidial oocysts were found in the faeces of some of the calves.

Further parasitological results are given in Tables 12.2 and 12.4, and the results of the measurements on the flukes are recorded in Table 12.3.

Most of the calves were slaughtered before the infection became patent. B458 had a count of 1 e.p.g. when slaughtered 12 weeks after infection, while B502 had counts of 3 e.p.g. at 13 weeks, 28 e.p.g. at 14 weeks and 51 e.p.g. at 15 weeks after infection when it was slaughtered. B456, the only other calf in which the infection became patent, had counts of 1 e.p.g. at 12 weeks, 3 e.p.g. at 13 weeks, 21 e.p.g. at 14 weeks, 48 e.p.g. at 15 weeks and 53 e.p.g. at 16 weeks post-infection. The sedimentation method was used for all these egg counts.

Although Fasciola eggs had not been detected in the faeces of B449, eggs were recovered from the gall bladder when it was slaughtered 86 days after infection.

The low recovery rates of F. gigantica in the 4 and 5 week old infections are probably due to technical difficulties as the flukes are very small at this time. The mean percentage recovery rate of flukes from those calves which had been infected for 8 weeks or more was 62.4±2.9 in Group 1 and 58.1±11.1 in

Table 12.2

Some experimental details

	B461	B454	B460	B457	B451	B452	B453	B458	B449	B459	B456	B502
Infective dose of metacercariae	-	1000	2000	-	1000	2000	-	1000	2000	-	1000	2000
No. of *F. gigantica* recovered	-	167	596	-	590	944	-	640	1156	-	642	1387
% recovery	-	16.7	29.8	-	59.0	47.2	-	64.0	57.8	-	64.2	69.4
Prepatent period (days)	-	-	-	-	-	-	-	82	>86	-	84	90
Experimental period (weeks)	4	4	5	8	8	8	12	12	12	16	16	15
*Weight of liver (g.)	3255	3223	3382	3867	3370	3988	3739	4667	5212	4437	7648	10085
Weight of hepatic lymph nodes (g.)	-	-	-	-	-	-	-	-	-	16	232	387
Volume of bile (ml.)	80	15	77	24	99	34	76	120	90	110	96	922
F. gigantica eggs in gall bladder	-	0	0	-	0	0	-	8250	2060	-	728500	115000

* Including gall bladder, bile and hepatic lymph nodes.

Table 12.3

Growth in F. gigantica (lengths in mm)

Calf No.	Group	Experimental period (weeks)	Area of liver	No. measured	Mean	S.D.	Range
B454	1	4	1	9	2.4	0.68	1.5-3.5
			2	50	2.3	0.56	1.5-3.5
			3	33	2.5	0.69	1.5-4
			Total	92	2.4		1.5-4
B460	2	5	1	69	3.3	0.91	1.5-6
			2	105	2.9	0.91	1.5-5.5
			3	57	2.6	0.88	1.5-4.5
			Total	231	2.9		1.5-6
B451	1	8	1	116	8.0	2.20	4-14
			2	86	7.6	2.22	3.5-12.5
			3	73	7.5	2.15	3.5-12
			Total	275	7.7		3.5-13
B452	2	8	1	158	6.4	3.20	4-15
			2	127	7.2	2.36	3-14
			3	128	7.9	2.27	2-15
			Total	413	7.1		2-15
B458	1	12	1	131	21.0	4.91	8-37
			2	139	23.5	4.85	12-41
			3	105	20.3	4.15	5-34
			Total	375	21.7		5-41
B449	2	12	1	292	21.6	4.79	4-33
			2	270	20.3	3.57	6-30
			3	150	20.3	3.24	7-27
			Total	712	20.8		4-33
B502	2	15	1	353	27.9	3.60	19-42
			2	259	27.9	4.07	16.5-40.5
			3	189	27.7	4.16	11-44
			Total	815	28.0		11-44
B456	1	16	1	91	35.3	6.81	23-56
			2	237	38.1	6.64	21-52
			3	65	32.0	5.18	23-44
			Total	402	36.6		21-56

Note: In some cases total figures include small additional numbers of flukes from the small intestine and gall bladder.

those in Group 2, thus there was little difference at this level of infection.

Ross, Todd and Dow (1966) have shown that in calves the parenchymal stages of Fasciola hepatica are preferentially found in the ventral portion of the liver (Area 3). No such preference was shown in the present studies (Table 12.4).

Nine flukes were found in the gall bladder of both B502 and B456, while there were 6 in the small intestine of the former and 16 in that of B456. Nine flukes were also recovered from the common bile duct of B502.

The possible effect of the infections with Eperythrozoa and Haemonchus on the interpretation of the haematological results from B502 will be referred to later.

Other parasitic infections: Moniezia were found in B452, B454, B457, B460 and B461, and Cysticercus bovis in B449, B454, B457 and B460. Also small numbers of adult Dictyocaulus viviparus were recovered from B449, B453, B454 and B460.

3. Post-mortem examinations

Detailed post-mortem examinations were carried out on all calves. The liver weights and certain other details are given in Table 12.2. The livers were enlarged in the animals which had been infected for 12 weeks or more and the hepatic lymph nodes were also very much enlarged in the later infections.

Table 12.4

Distribution of F. gigantica in the liver

Calf No.	Group	Period (weeks)	Area 1 (dorsal) No. of flukes	Area 1 (dorsal) Wt. of liver(g.)	Area 1 (dorsal) Ratio	Area 2 (central) No. of flukes	Area 2 (central) Wt. of liver(g.)	Area 2 (central) Ratio	Area 3 (ventral) No. of flukes	Area 3 (ventral) Wt. of liver(g.)	Area 3 (ventral) Ratio
B460	2	5	184	1134	1:6.2	241	1362	1:5.7	171	851	1:5.0
C36	-	6	235	1287	1:5.5	122	1135	1:9.3	107	660	1:6.2
B451	1	8	199	1149	1:5.8	224	1256	1:5.6	167	808	1:4.8
B452	2	8	320	1327	1:4.1	351	1433	1:4.1	273	1043	1:3.8
C32*	-	10	258	1571	1:6.1	166	1215	1:7.3	84	687	1:8.2
B458	1	12	231	1534	1:6.6	227	1714	1:7.6	182	1023	1:5.6
B449	2	12	462	1652	1:3.6	442	1970	1:4.5	252	1152	1:4.6
B502	2	15	560	3000	1:5.4	460	3179	1:6.9	344	2375	1:6.9
B456	1	16	159	2337	1:14.7	332	3362	1:10.1	125	1475	1:11.8

* 51 additional flukes were recovered from the liver of this calf but the area was not recorded.

The liver areas are as described in Ross, Todd and Dow (1966).

Experimental details of C32 and C36 are given in Chapter 14.

There were no signs of flukes in any of the control animals, nor any evidence that they had ever been infected. B457, B459 and B461 appeared entirely normal but B453 had a well encapsulated abscess in the liver which was found to be sterile on culture.

Slight fibrinous adhesions were seen on the pleura of most of the calves.

B502 (Group 2) was the only calf in poor bodily condition. Very little fat was present and this was rather gelatinous. There was also some evidence of jaundice, and petechial haemorrhages were present on both kidneys.

Full details of liver lesions will be given in Chapter 15. There were no marked differences from the lesions described there.

4. Biochemical data

As it was not possible to carry out the biochemical tests on fresh sera the sera were stored at -25°C. The results of the tests should be interpreted with this in mind.

(a) Iodine flocculation tests: The results of these tests, carried out on sera stored for 12-15 months, are shown in Table 12.5. The control calves, and all sera taken preinfection and up to 3 weeks post-infection, were negative. The 2 minute reading was taken in every case as it was found to give more reliable results than the reading taken after 30 minutes. Each test was carried out with a serum/

reagent ratio of 1:1, 2:1 and 4:1.

Table 12.5

Iodine flocculation tests

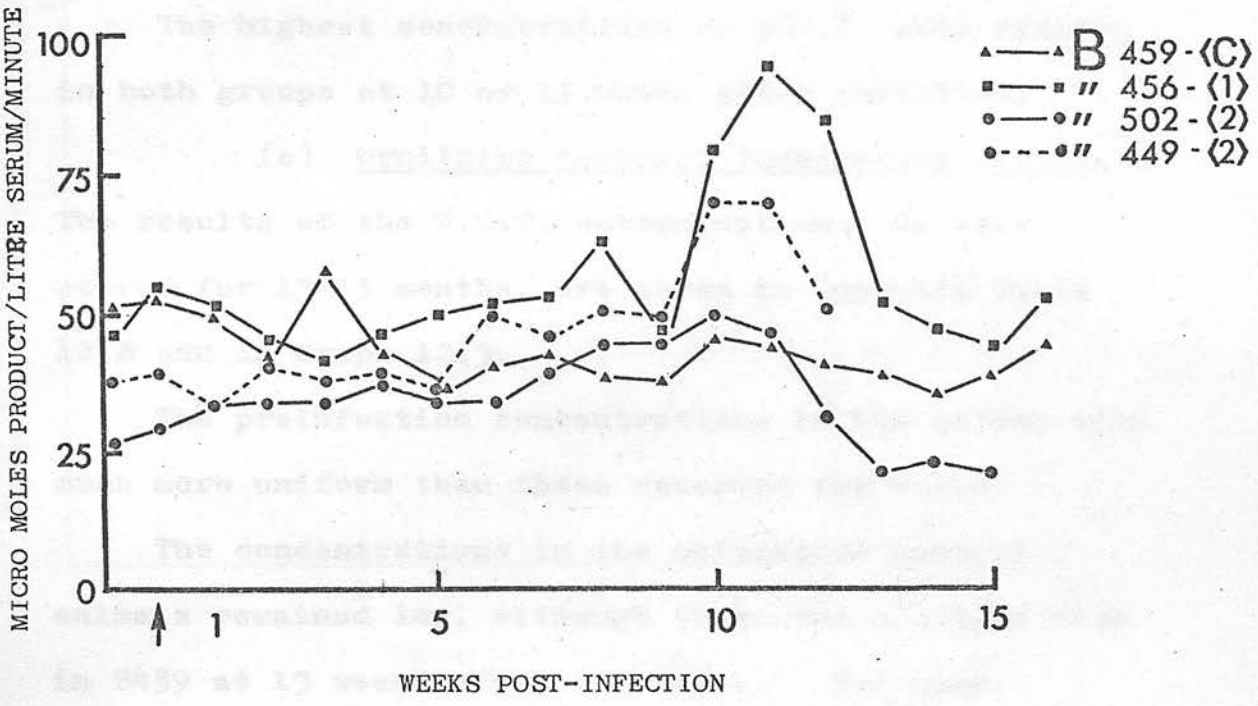
Calf No.	Group	Weeks post-infection													
		3	4	5	6	7	8	9	10	11	12	13	14	15	16
B449	2	0	0	0	0	1	1	1	0	0	12				
B451	1	0	0	0	4	0	0								
B452	2	1	0	±	0	0	4								
B454	1	0	0												
B456	1	0	0	0	0	0	0	0	0	0	2	12	8	8	4
B458	1	0	0	0	0	0	0	0	0	0	1				
B460	2	0	0	0											
B502	2	0	1	0	0	0	±	±	0	0	12	12	3	6	

± = doubtful result. The results are scored as in Chapter 9.

Positive results were obtained in the two calves in Group 2 by 12 weeks post-infection and in one animal in Group 1 by 13 weeks after infection. The remaining animal in Group 1 had not become positive when slaughtered at 12 weeks post-infection.

Occasional positive results occurred before 12 weeks after infection which probably indicates that a score of more than 4 is necessary to establish a positive result under these conditions.

(b) Serum glutamic exaloacetic transaminase (S.G.O.T.): The results of the S.G.O.T. determinations, on sera stored for 9 months, are shown in Appendix Table 12.B and Graph 12.2. Some calves



GRAPH 12.2 SERUM GLUTAMIC OXALOACETIC TRANSAMINASE

had much higher preinfection concentrations than others and in the case of B451 one of these was nearly as high as the highest recorded in any of the infected calves.

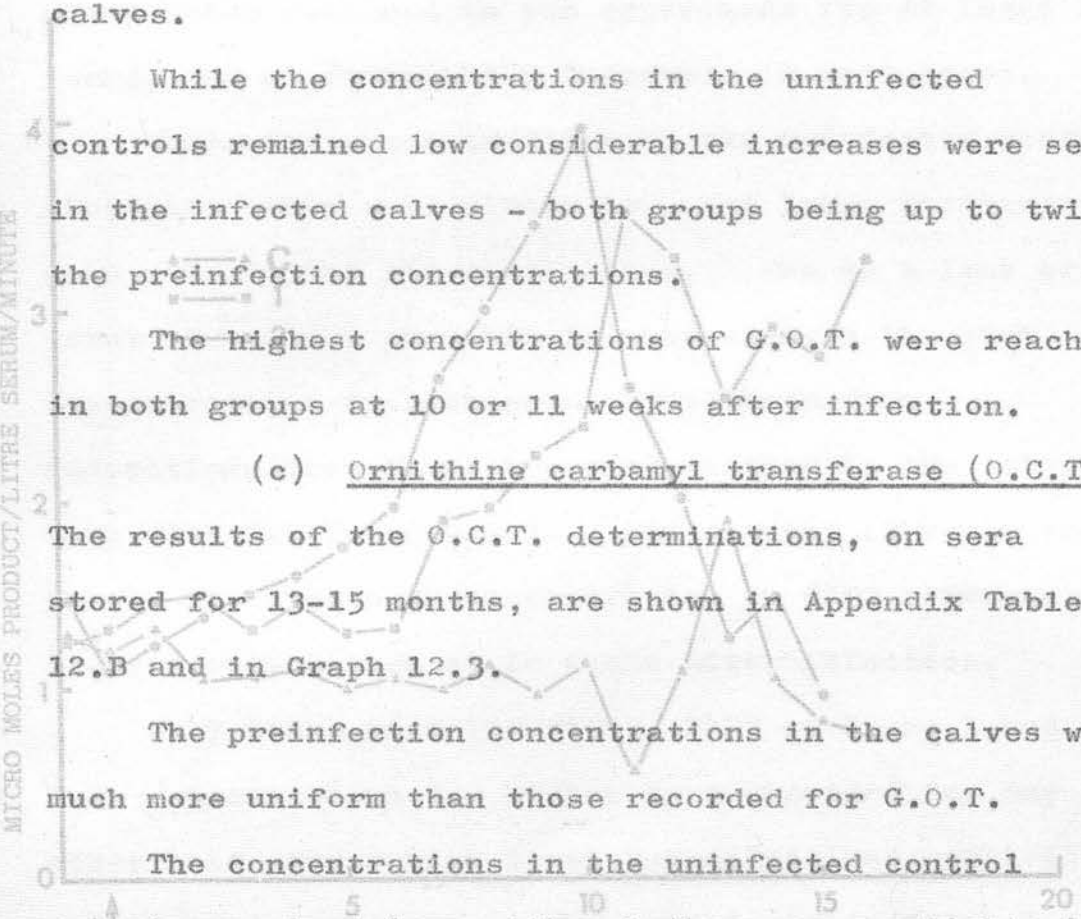
While the concentrations in the uninfected controls remained low considerable increases were seen in the infected calves - both groups being up to twice the preinfection concentrations.

The highest concentrations of G.O.T. were reached in both groups at 10 or 11 weeks after infection.

(c) Ornithine carbamyl transferase (O.C.T.): The results of the O.C.T. determinations, on sera stored for 13-15 months, are shown in Appendix Table 12.B and in Graph 12.3.

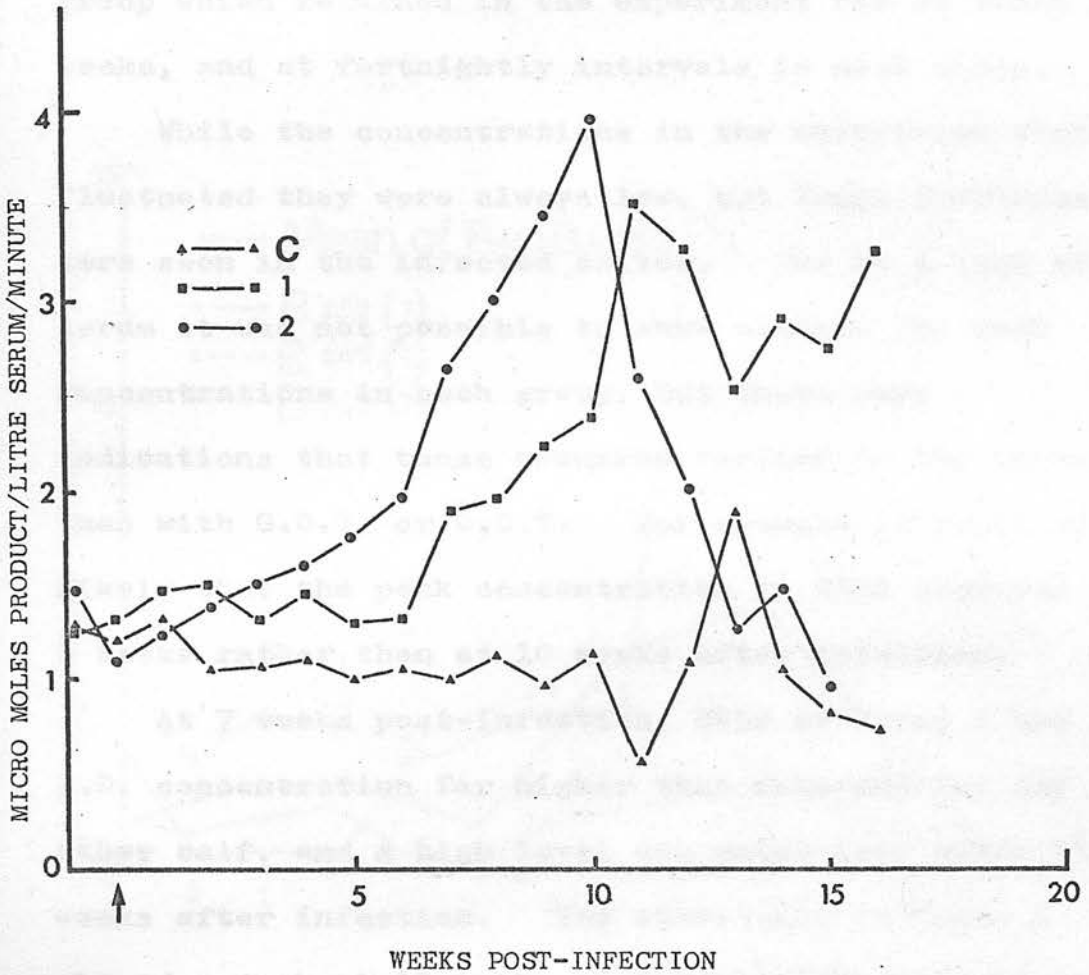
The preinfection concentrations in the calves were much more uniform than those recorded for G.O.T.

The concentrations in the uninfected control animals remained low, although there was a slight rise in B459 at 13 weeks after infection. The mean concentration in Group 2 was highest at 10 weeks after infection falling sharply thereafter, reaching normal levels in B502 by 13 weeks after infection. In Group 1 the peak level was reached at 11 weeks post-infection, and tended to remain relatively high. The highest concentrations were of the same order in each group.



GRAPH 12.3 ORNITHINE CARBAMYL TRANSFERASE

(d) Sorbitol dehydrogenase (S.D.): The results of the S.D. determinations, on sera stored for



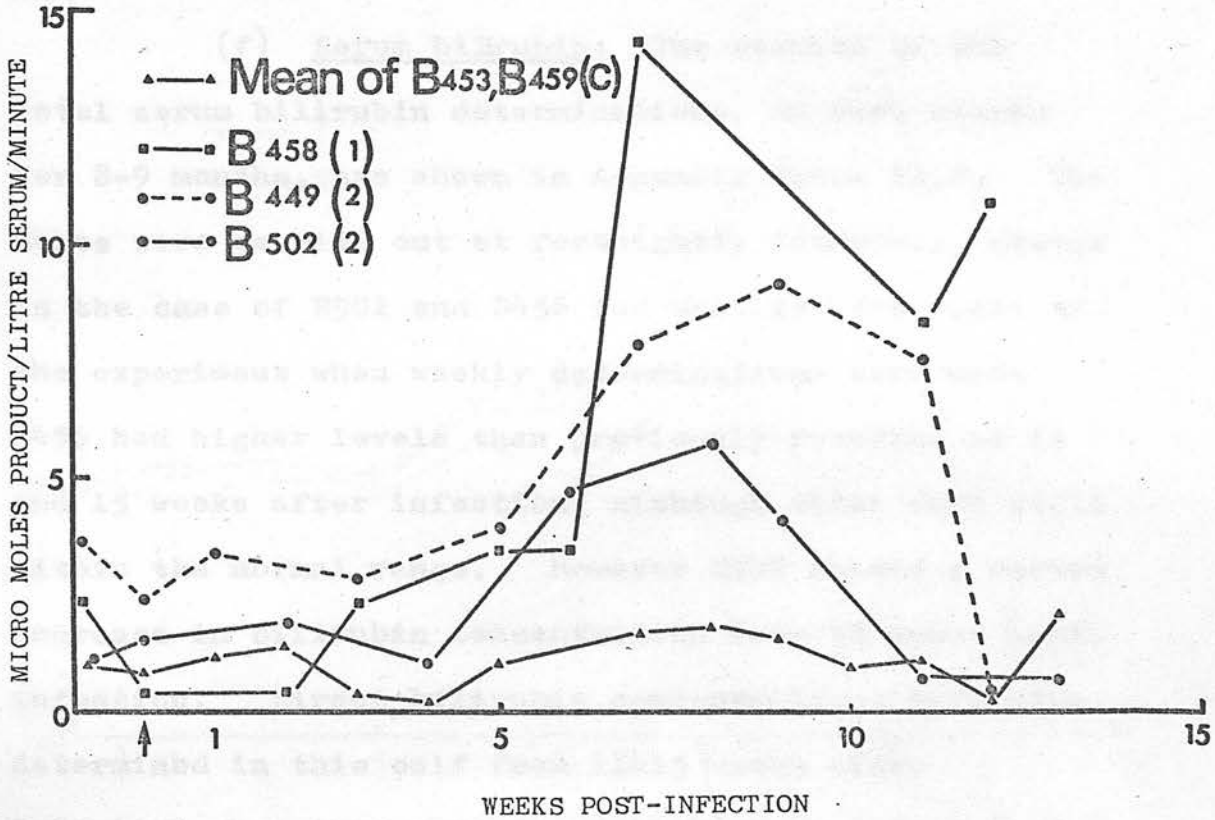
GRAPH 12.3 ORNITHINE CARBAMYL TRANSFERASE

24-27 months, are shown in Appendix Table 12.B, and some selected results are shown in Graph 12.4. The tests were carried out on only the two animals in each group which remained in the experiment for at least 12 weeks, and at fortnightly intervals in most cases.

While the concentrations in the uninfected controls fluctuated they were always low, but large increases were seen in the infected calves. Due to a lack of serum it was not possible to show clearly the peak concentrations in each group, but there were indications that these occurred earlier in the infection than with G.O.T. or O.C.T. For example it would seem likely that the peak concentration in B502 occurred at 8 weeks rather than at 10 weeks after infection.

At 7 weeks post-infection, B458 of Group 1 had an S.D. concentration far higher than recorded for any other calf, and a high level was maintained until 12 weeks after infection. The other calf in Group 1 showed a peak at 10 weeks post-infection followed by a fall to preinfection levels as occurred in the more heavily infected animals.

(e) Alkaline phosphatase: The results of the alkaline phosphatase determinations, on sera stored for 18 months, are shown in Appendix Table 12.B. There were no marked differences between the infected and control calves during the course of the experiments, wide fluctuations round the preinfection levels being shown. However the levels found in B502,



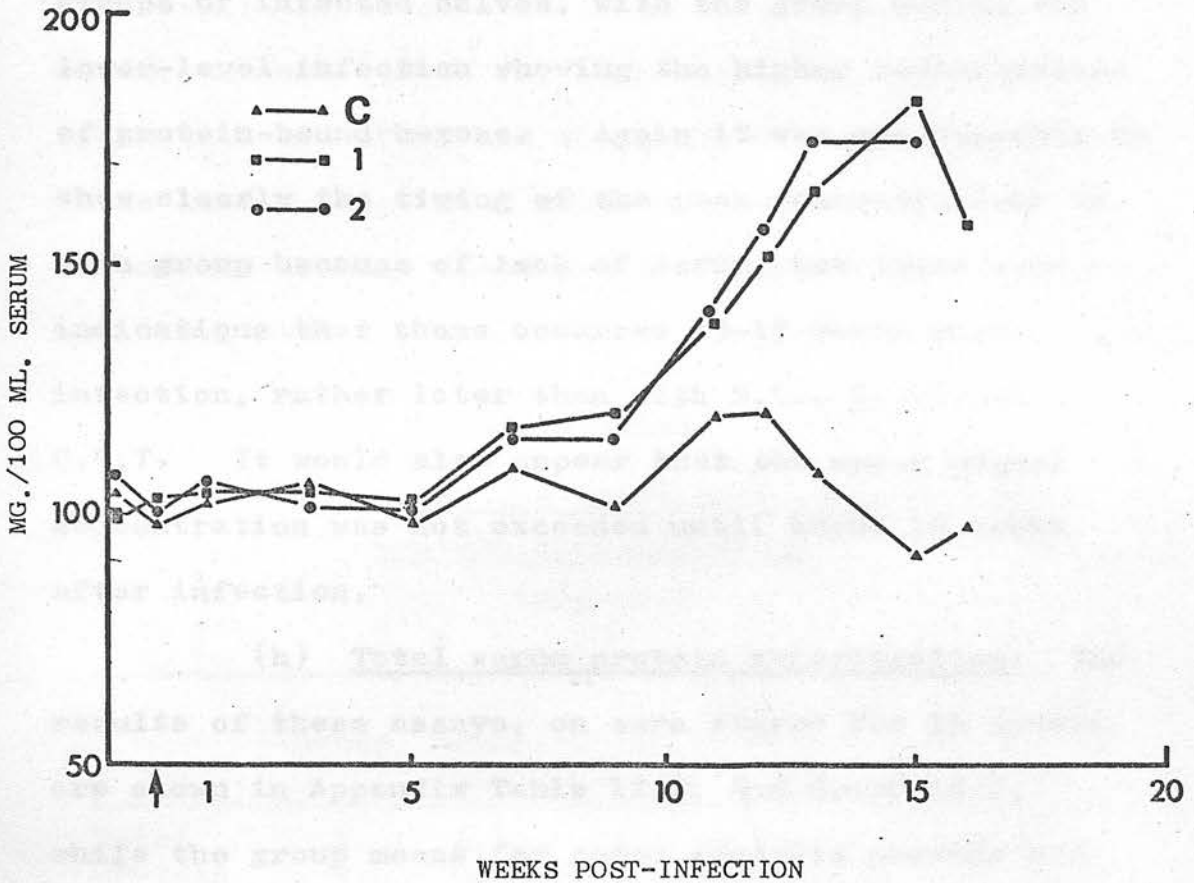
GRAPH 12.4 SORBITOL DEHYDROGENASE

which were low throughout the experiment, fell to even lower levels from 9 weeks after infection. Preinfection concentrations were much higher in B451 than in any of the other calves and they tended to remain high. High preinfection G.O.T. concentrations had also been found in this calf.

(f) Serum bilirubin: The results of the total serum bilirubin determinations, on sera stored for 8-9 months, are shown in Appendix Table 12.C. The tests were carried out at fortnightly intervals, except in the case of B502 and B456 for the last few weeks of the experiment when weekly determinations were made. B456 had higher levels than previously recorded at 14 and 15 weeks after infection, although these were still within the normal range. However B502 showed a marked increase in bilirubin concentration from 12 weeks post-infection. Direct bilirubin concentrations were also determined in this calf from 11-15 weeks after infection when they were found to be 0.2, 0.3, 0.3, 0.4 and 2.0 mg./100 ml. respectively.

The total bilirubin concentrations in all the other calves remained at or below those found before infection.

(g) Protein-bound hexose: The results of these determinations, on sera stored for 12-16 months, are shown in Appendix Table 12.C and Graph 12.5. The assays were carried out only on the two calves in each group which remained in the experiment for at least 12



GRAPH 12.5 PROTEIN-BOUND HEXOSE

weeks and at fortnightly intervals in most cases.

In only one instance did the concentration in the control calves rise above the upper normal limit of 120 mg./100 ml. serum, and then only very slightly. However large increases were seen in the level in both groups of infected calves, with the group having the lower-level infection showing the higher concentration of protein-bound hexose. Again it was not possible to show clearly the timing of the peak concentrations in each group because of lack of serum, but there were indications that these occurred 13-15 weeks post-infection, rather later than with S.D., G.O.T. or O.C.T. It would also appear that the upper normal concentration was not exceeded until about 10 weeks after infection.

(h) Total serum protein concentration: The results of these assays, on sera stored for 15 months, are shown in Appendix Table 12.D, and Graph 12.8, while the group means for three separate periods are shown in Table 12.6.

The tests were carried out at fortnightly intervals in most cases, and only on those two calves from each group which remained in the experiment for at least 12 weeks.

While the total serum protein concentration in the control calves remained below preinfection levels increases were seen in all the infected ones. B456 had a very high concentration at 15 weeks after

infection but this had fallen to more normal levels by the next week. The preinfection levels in both the infected groups were not exceeded until 11-12 weeks after infection.

(i) Serum protein fraction concentrations:

The results of these studies by electrophoresis of serum, on sera stored for 18 months, are shown in Appendix Table 12.D, and the group means are shown in Table 12.6. Fig. 12.1 shows a representative Chromoscan tracing (B458 preinfection) to indicate the empirical method used for defining the protein fractions.

FIG. 12.1 CHROMOSCAN TRACE OF PREINFECTION SERUM FROM B458

Gamma Globulin Beta Globulin Alpha Globulin Albumin

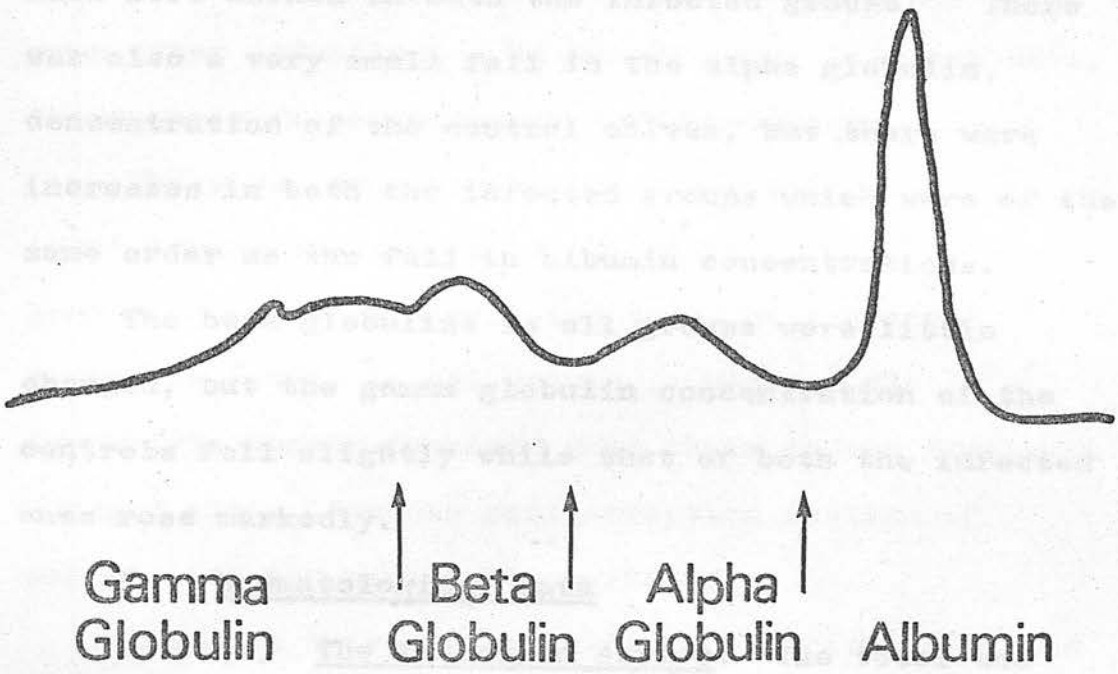
Table 12.6

Mean total and fractional serum protein concentrations

(g%±s.d.)

	Group	Preinfection	Weeks Post-infection 5-9	11-16
Total Protein	1	6.40±0.32	5.83±0.17	7.42±1.05
	2	6.60±0.26	6.32±0.17	7.12±0.60
	C	6.40±0.42	5.80±0.22	6.0 ±0.43
Albumin	1	2.32±0.15	1.95±0.10	1.90±0.27
	2	2.25±0.13	2.02±0.18	1.80±0.19
	C	2.23±0.15	1.94±0.17	2.08±0.23
Alpha Globulin	1	1.38±0.15	1.40±0.09	1.97±0.27
	2	1.40±0.16	1.45±0.19	1.82±0.31
	C	1.40±0.12	1.34±0.15	1.32±0.13
Beta Globulin	1	0.95±0.26	0.85±0.19	1.05±0.17
	2	0.85±0.05	0.87±0.13	0.96±0.09
	C	0.95±0.13	0.80±0.22	0.88±0.09
Gamma Globulin	1	1.78±0.22	1.62±0.15	2.47±0.51
	2	2.03±0.21	1.95±0.13	2.52±0.31
	C	1.88±0.22	1.68±0.17	1.64±0.27

FIG. 12.1 CHROMOSCAN TRACE OF PREINFECTION SERUM FROM B458



The electrophoretic pattern of all the sera was the same in respect of albumin, alpha and beta globulin, but the irregularities in the trace from the fraction designated gamma globulin precluded further subdivision.

There was a slight fall in the mean concentration of albumin in the control calves but these falls were much more marked in both the infected groups. There was also a very small fall in the alpha globulin concentration of the control calves, but there were increases in both the infected groups which were of the same order as the fall in albumin concentrations.

The beta globulins in all groups were little changed, but the gamma globulin concentration of the controls fell slightly while that of both the infected ones rose markedly.

5. Haematological data

(a) The leucocyte series: The total and differential leucocyte counts are shown in Appendix Table 12.E. In the control calves there was a slight increase, followed by a fall at 7-9 weeks after the infection date to uniformly lower levels. However, in both the infected groups there was a progressive increase to 6 weeks after infection. This was followed by a fall and then a very marked leucocytosis which reached its maximum level in the period 12-14 weeks post-infection, after which the counts fell again.

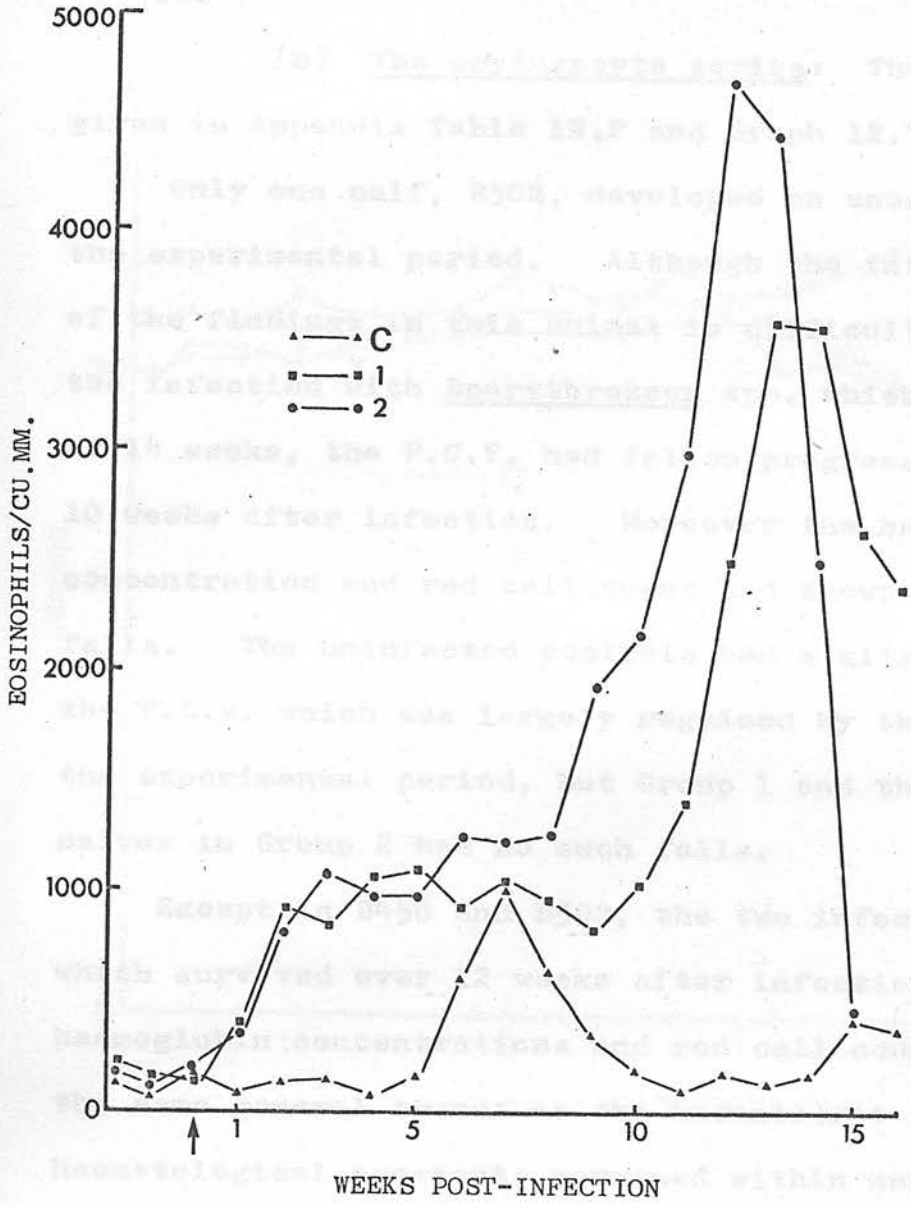
The eosinophil counts are shown in Graph 12.6. There were low eosinophil counts in the control calves except from 6-8 weeks after the infection date when B457 and B459 had a mild eosinophilia. All the calves in the infected groups had a very marked eosinophilia which tended to parallel the total leucocyte counts. Most of the calves had gradual rises, which were often evident as early as the first week after infection. In two animals, B451 and B460, these early rises were much more pronounced.

There was considerable weekly variation in the total lymphocyte counts in all three groups, but in general the levels paralleled those of the total leucocyte counts in all groups. However the lymphocyte counts remained near their former levels in Group 2 after 11 weeks post-infection instead of paralleling the marked leucocytosis.

Although there was considerable variation in the total neutrophil counts of all three groups of calves there were no significant changes, except in B502 (the sole survivor from Group 2) which had a very marked rise from 12 weeks after infection.

Monocytes and basophils showed no significant trends in any of the calves. Band neutrophils were rarely seen except in B502 where they were associated with the marked neutrophilia from 12 weeks post-infection.

No toxic granulation or other indications of



GRAPH 12.6 EOSINOPHIL COUNTS

toxaemia were seen in the neutrophils of the infected calves.

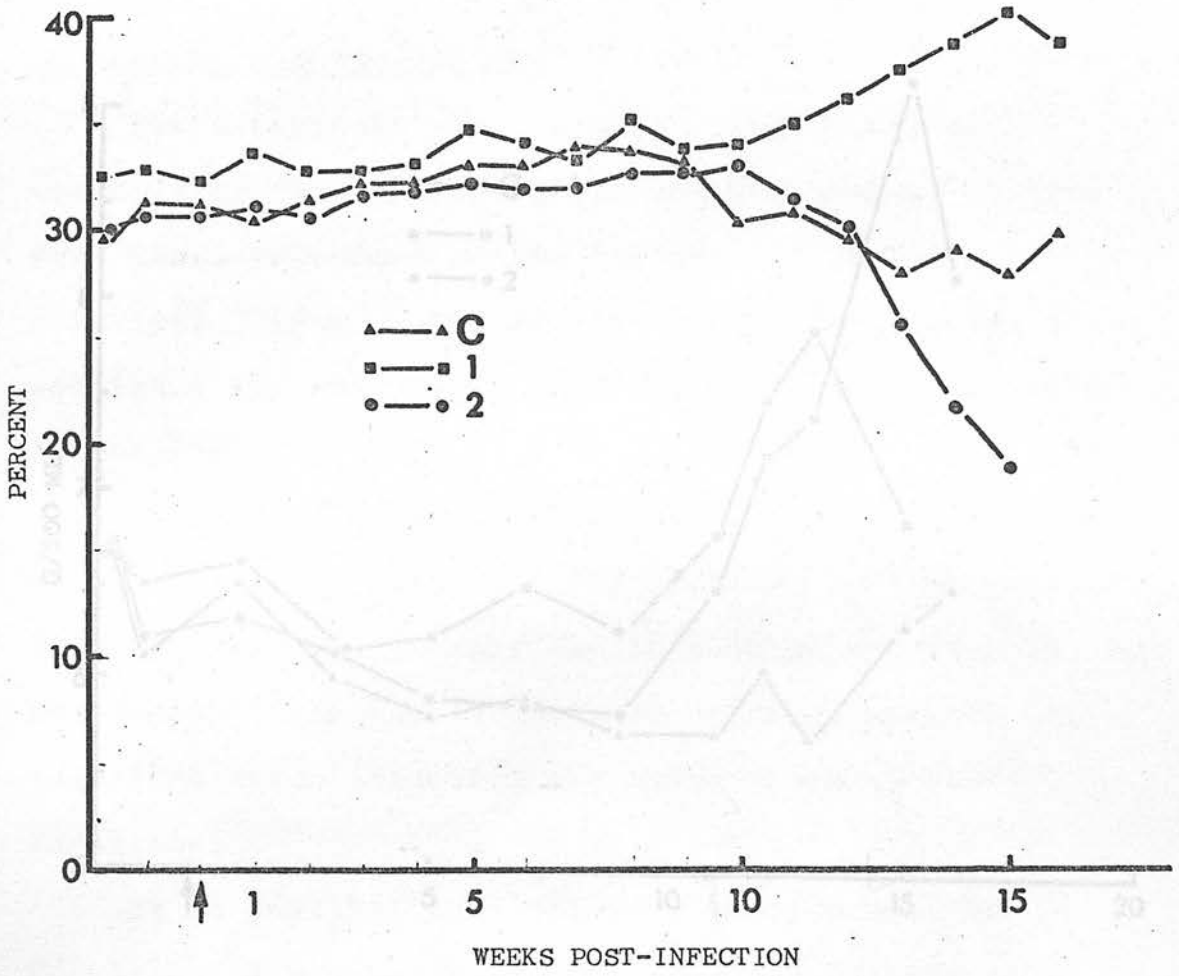
(b) The erythrocyte series: The results are given in Appendix Table 12.F and Graph 12.7.

Only one calf, B502, developed an anaemia during the experimental period. Although the interpretation of the findings in this animal is difficult, in view of the infection with Eperythrozoon spp. which was found at 14 weeks, the P.C.V. had fallen progressively from 10 weeks after infection. Moreover the haemoglobin concentration and red cell count had shown similar falls. The uninfected controls had a slight fall in the P.C.V. which was largely regained by the end of the experimental period, but Group 1 and the other calves in Group 2 had no such falls.

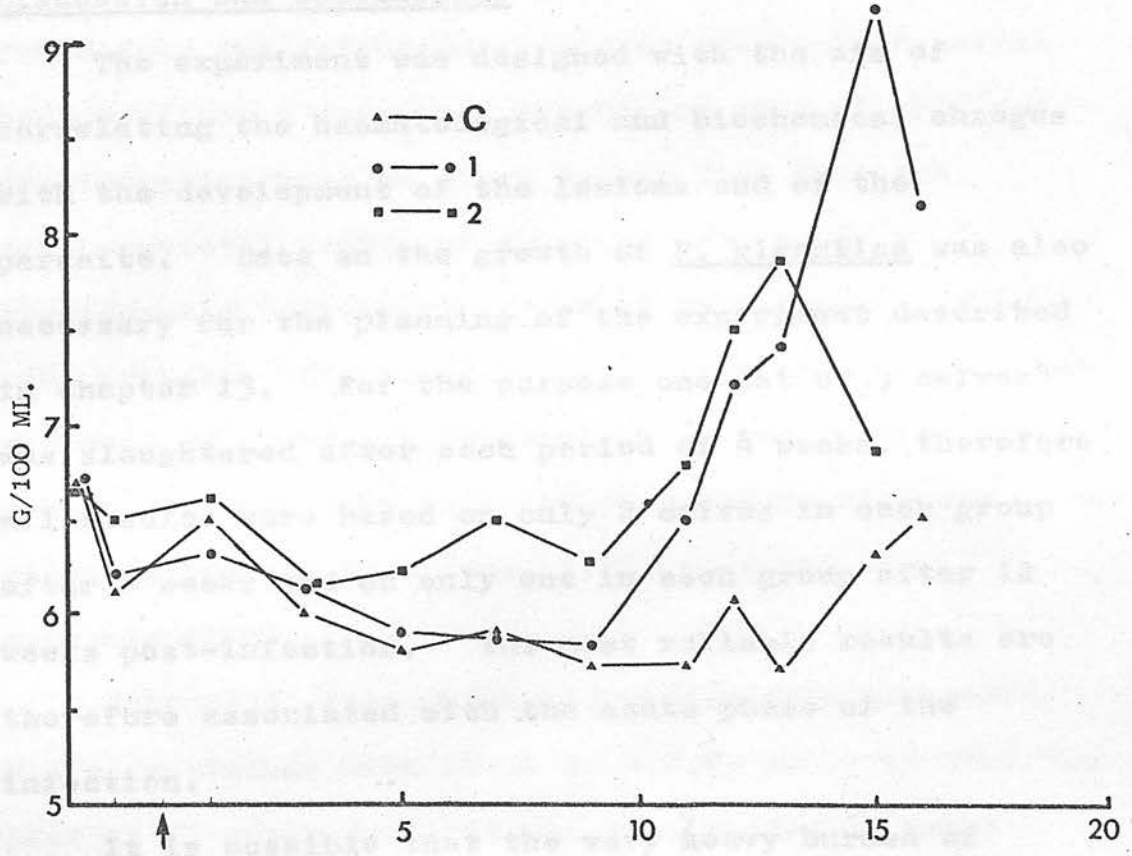
Except in B456 and B502, the two infected calves which survived over 12 weeks after infection, the haemoglobin concentrations and red cell counts showed the same general trends as the haematocrit so that the haematological constants remained within normal limits. In these two animals, however, the mean corpuscular volume increased from the 13th week after infection, the terminal figures being much higher than those preinfection or at 12 weeks after infection.

Slight increases in the mean corpuscular haemoglobin (M.C.H.) were also shown by B456 and B502.

No abnormalities were seen in the erythrocytes of any calf during the course of the experiment and no



GRAPH 12.7 PACKED CELL VOLUME



GRAPH 12.8 TOTAL SERUM PROTEIN

immature cells were seen. Moreover no parasites were found in blood smears from any calf, except for the single record of Eperythrozoon spp. in B502 at 14 weeks post-infection.

Discussion and conclusions

The experiment was designed with the aim of correlating the haematological and biochemical changes with the development of the lesions and of the parasite. Data on the growth of F. gigantica was also necessary for the planning of the experiment described in Chapter 13. For the purpose one lot of 3 calves was slaughtered after each period of 4 weeks, therefore all results were based on only 2 calves in each group after 8 weeks and on only one in each group after 12 weeks post-infection. The most reliable results are therefore associated with the acute phase of the infection.

It is possible that the very heavy burden of F. gigantica had rendered B502 more susceptible to infection with Haemonchus spp. as the numbers recovered from this calf were very much higher than in any other calf. It is also possible that the higher counts of gastro-intestinal nematode eggs, which were recorded in the faeces of the more heavily infected calves towards the end of the experimental period, may also have been due to a lowered resistance to these parasites because of the F. gigantica infection.

The anaemia which developed in B502 may have been

accentuated by the heavy infection of Haemonchus spp. Further, the infection with Eperythrozoon spp., although only seen first at 14 weeks post-infection, may have also increased the degree of the anaemia which was already developing. Although Neitz (1940) found that the infection of non-splenectomised calves caused either a very mild anaemia or none at all, due consideration must be given to the fact that the erythropoietic system of this calf was under considerable additional stress at this time. It is also probable that 'Spirotrypan forte' (Hoechst), which was used to treat this infection, caused further liver damage although only half the recommended dose was given (Kühne and Schneider, 1966, personal communication).

Calf B456, from which 642 flukes were recovered, had a continuous high level of O.C.T. activity from the peak at 11 weeks until it was slaughtered 16 weeks after infection. This is a surprising result as the other biochemical parameters had all recorded falls at this time. Also the haematological findings were within a normal range, except the eosinophil count which was still high although falling. Furthermore at the post-mortem examination no lesions were found in the small intestine, the only organ besides the liver which has a high O.C.T. activity (Ford, 1965). Moreover it was found that most of the flukes had passed out of the liver parenchyma into the bile ducts

and that the acute phase of the disease was over, and this view was confirmed on examination of the numerous photographs of transverse sections of the liver. Also, at this time the F. gigantica egg count was 53 e.p.g. faeces. Certainly, in B502, which had twice the number of flukes in the liver, the O.C.T. concentration had fallen to preinfection levels at this time.

The three enzymes assayed all showed large increases in concentration in the serum of the infected animals, this being associated with liver damage, in spite of the long storage period at -25°C.

There were only small differences between the serum enzyme concentrations and some other parameters in Groups 1 and 2 in spite of the presence of twice as many flukes in the livers of the latter group. This is explicable if the damage to the liver cells, which arises from the host reaction to the presence of the parasites, is also considered. Dawes (1963) thought the liver damage much too substantial to be attributable to the direct feeding activities of the flukes and found widespread breakdown of hepatic tissue round the fluke tracks as a result of inordinately large leucocytic infiltration. Such a host response might be relatively independent of the numbers of parasites present over a threshold level, and would also take some weeks to develop.

Total leucocyte counts gradually increased in the

infected calves up to 11 weeks after infection, and then rose rapidly to high levels, before falling after 14 weeks post-infection. Although eosinophilia was very marked in all the infected calves, and contributed to some of the leucocytosis, there was also a marked lymphocytosis from 12 weeks post-infection in one calf and a very marked neutrophilia in another from week 11.

The high levels of G.O.T. and alkaline phosphatase which were found in the serum of B451 around the time of infection may have been due to lesions in any of a range of different tissues or organs (Boyd, 1962; Cornelius, Bishop, Switzer and Rhode, 1959). But these lesions were not in the liver or small intestine because the O.C.T. levels at this time were low and of the same order as those in the other calves before infection (Ford, 1965). No lesions were found at the post-mortem examination to account for the high preinfection G.O.T. and alkaline phosphatase levels.

CHAPTER 13

Chronic Fascioliasis in Cattle (Fasciola gigantica): Single and Challenge Infections

Introduction

Single infections at a lower level than in Chapter 12 were studied in the same detail but over a longer period. Also the effects of a challenge infection were compared with the single infection using the same parameters as before. Ross (1967) reported the induction of an acquired self-cure in cattle by a challenge infection with Fasciola hepatica and an attempt was made to confirm this finding with F. gigantica. Evidence of an acquired immunity was also looked for, in the challenge infections when compared with single infections of the same size, the parameters considered being the lengths and numbers of flukes which were recovered.

When this work was carried out in 1968 the only published work on experimental challenge infections with F. gigantica in cattle was by Sewell (1966), although Bitakaramire (1969) has since published the results of his experiment.

Experimental design

(a) Animals

Fourteen Guernsey-type castrated male calves were obtained from a farm with no recent history of fascioliasis and maintained as previously described.

The calves were 3 months of age on arrival. A further 6 calves of similar age and type were obtained from the same farm; 2 of these were 3 months old on arrival and 4 were 9 months old. These 6 calves were found to be infected with Anaplasma marginale and/or Babesia bigemina and were kept separate from the other 14 (Chapter 8).

(b) Treatments

The experiment began when all the calves were 12-13 months of age. Three groups of 4 calves were selected at random from the batch of 14, to form Groups 1, 2 and 3, while Group 4 was selected at random from the batch of 6. The remaining 4 calves formed the uninfected control Group C. The infection details are shown in Table 13.1. All the calves were slaughtered 18 weeks after the beginning of the experiment.

The period of slaughter extended over 8 days, one calf from each group being slaughtered on each day. F. gigantica had been shown to grow rapidly from 8-10 weeks after infection (Chapter 14), but this procedure compensated for the growth of flukes which took place during this period of 8 days.

Detailed results are usually only given for Groups 1, 2 and the 2 associated animals in Group C (B507 and B511); unless the results from Groups 3 and 4 materially alter the findings from the similar infections in Group 1 in this experiment, and Group 1

in the experiment described in Chapter 12. Likewise, Group C refers to B507 and B511 only unless specifically stated.

Table 13.1

Infection details

Group	Calf No.	No. of metacercariae given at weeks 0	10
1	B506 B509 B512 B513	500	0
2	B498 B500 B501 B504	500	500
3	B499 B503 B505 B510	0	500
4	B477 B448 B875 B968	0	1000
C	B507 B511 C28 C37	0	0

The metacercariae used to infect these calves were from $6\frac{1}{2}$-$7\frac{1}{2}$ days old. To obviate any possible differences in infectivity rates of metacercariae from different batches of snails, the infested pieces of grass were cut short and randomised before counting started. However, Urquhart (1954) found that

cercariae of Fasciola hepatica from different snails showed no consistent differences in their infectivity in rabbits.

(c) Observations

From the results described in Chapter 12 it had appeared very unlikely that erythrocyte values would be affected by these smaller infections and therefore determinations of these parameters were carried out at longer intervals. Total and differential leucocyte counts were carried out at weekly intervals.

Serum was collected weekly for use in biochemical studies. Nematode egg counts were made on faeces collected at the end of the experiment. The prepatent period for F. gigantica was determined and weekly egg counts were carried out thereafter.

The liver lesions were assessed immediately after the post-mortem examination, and the livers stored at -15°C. Later the flukes were recovered and all the undamaged ones measured.

The calves were weighed at weekly intervals.

All the above observations were also carried out on Groups 3 and 4 and the control animal, C28. Only live-weight determinations were carried out on the control animal C37.

Results

1. Clinical data

No clinical symptoms which could be attributed to

fascioliasis were seen in any of the calves. Immediately before the experiment started the calves in the batch of 6 all reacted to the capillary agglutination tests (Chapter 8), B448 reacting to A. marginale, B875, B968, C28 and C37 reacting to B. bigemina, and B447 to both. Nevertheless, the stress of infection with 1000 metacercariae of F. gigantica did not result in the breakdown of the premunity to these diseases in any of these animals in Group 4. However this group lost weight between 6 and 8 weeks after infection, whereas the 2 uninfected controls continued to gain weight (Appendix Table 13.A).

During the 5th and 6th weeks of the experiment, several calves developed slight upper respiratory infections which resolved without treatment.

Eperythrozoon sp. was seen in the weekly blood smears from 7 calves, including one control, between 6 and 9 weeks after the experiment began. The organisms were only seen on a single occasion in each animal and no clinical symptoms were observed. However, the infection was associated with a transient leucopenia, affecting all the leucocytes except the eosinophils, and in one animal (B509) may have caused a transient anaemia during which the P.C.V. fell to 20%, 9 weeks after infection with F. gigantica.

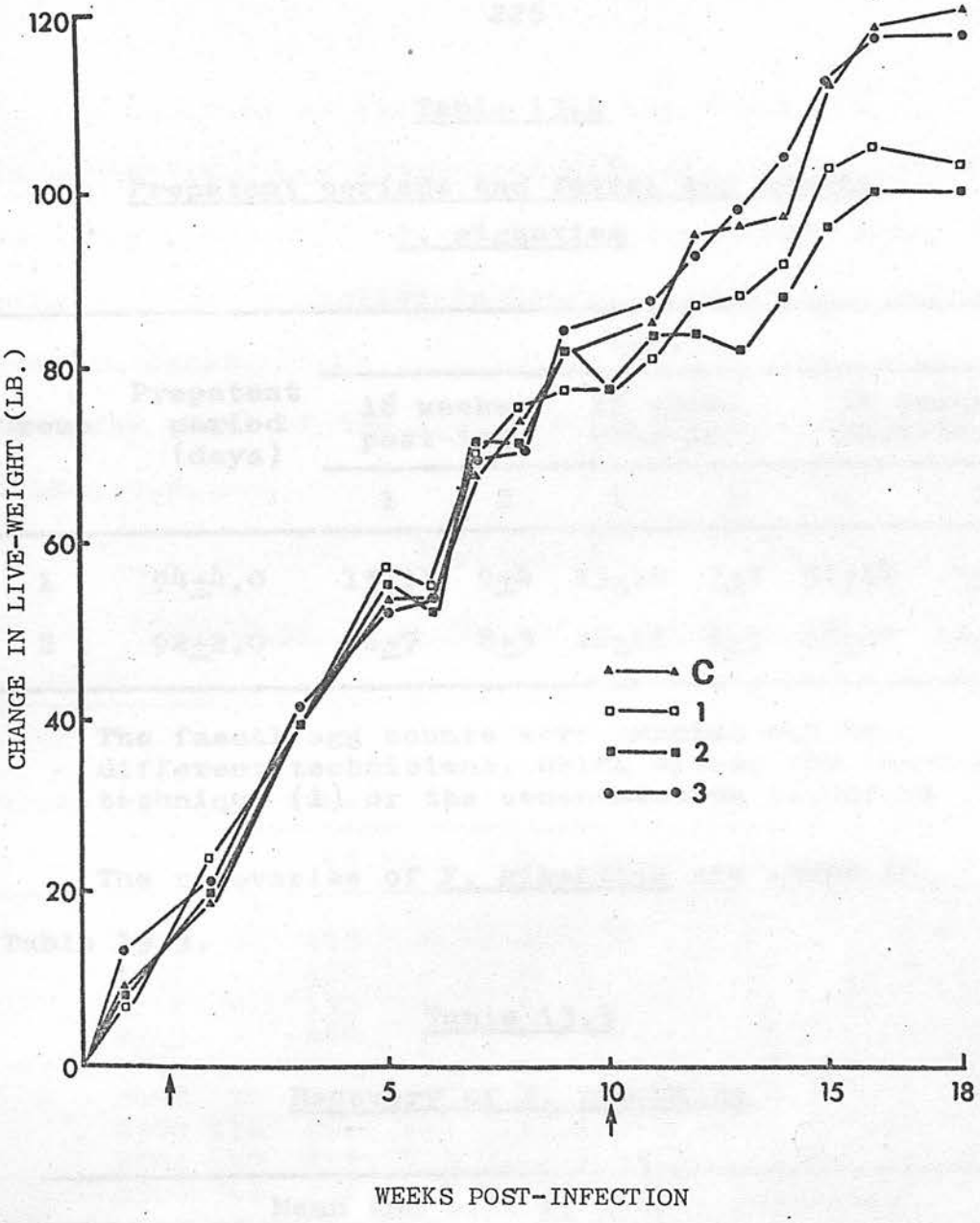
The live-weights of all calves are shown in Appendix Table 13.A and the means of Groups 1, 2, 3 and

C in Graph 13.1. The fall-off in live-weight gain about 9-10 weeks after infection, followed by a resumption at approximately the former rate, which had also been seen in the experiment described in Chapter 11 is clearly seen. The infection also appears to have reduced the overall weight gains of Groups 1 and 2 as compared with Group C ($F = 4.84$, $P = c.0.05$).

2. Parasitological data

No attempt was made to control gastro-intestinal nematodes by the use of anthelmintics. Nematode egg counts were carried out on faeces collected from each calf on two separate days towards the end of the experimental period. The mean counts were Group 1, 63 e.p.g.; Group 2, 32 e.p.g.; Group 3, 38 e.p.g.; Group 4, 44 e.p.g. and Group C (B507, B511 and C28), 42 e.p.g. There was thus little difference between the groups. Coccidial oocysts were also found in the faeces of 11 calves at this time, the highest count recorded being 450 oocysts per gram of faeces.

The prepatent periods and faecal egg counts of F. gigantica are recorded in Table 13.2. An analysis of variance showed no significant difference between Groups 1 and 2, only the results from the two methods and the weeks of faecal collections being significantly different ($P<0.001$ and $P<0.05$ respectively). There is no significant difference between the prepatent periods in the two groups.



GRAPH 13.1 LIVE-WEIGHTS

Table 13.2

Prepatent periods and faecal egg counts, F. gigantica

Group	Prepatent period (days)	E.P.G. 16 weeks post-inf.		17 weeks post-inf.		18 weeks post-inf.	
		1	2	1	2	1	2
1	94±4.0	15±11	9±4	23±10	7±3	32±18	9±7
2	92±2.0	16±7	8±3	22±11	8±5	33±25	16±11

The faecal egg counts were carried out by different technicians, using either the sieving technique (1) or the sedimentation technique (2)

The recoveries of F. gigantica are shown in Table 13.3.

Table 13.3

Recovery of F. gigantica

Group	Mean and s.d. of flukes recovered: 8 weeks post-inf. Number	%	18 weeks post-inf. Number	%
1	-	-	290±44	58±9
2	195±54	39±11	314±29	63±6
3	287±37	57±7	-	-
4	462±97	46±10	-	-

There is no significant difference between the recoveries of 18-week-old flukes from Groups 1 and 2 ($F \approx 1$). There is thus no evidence of any "acquired self-cure". There is however a significant reduction

in the recovery of the flukes of the challenge infection in Group 2 compared with the single infection in Group 3 ($F = 7.65$, $P < 0.05$), the recovery from the single larger infection in Group 4 being intermediate between these.

The sizes of the flukes recovered are shown in Table 13.4.

Table 13.4

Growth of F. gigantica (lengths in mm.)

Group	Calf No.	No. measured at weeks		Weeks post-infection: 8			18		
		8	18	Mean	S.D.	Range	Mean	S.D.	Range
1	B506	-	175	-	-	-	36.1	7.52	7.5*-52.5
	B509	-	184	-	-	-	36.9	6.08	23 -55
	B512	-	153	-	-	-	42.0	6.90	26 -56.5
	B513	-	260	-	-	-	44.2	7.56	25 -63
2	B498	59	179	9.0	3.16	3.5-16.5	38.0	7.47	24 - 54
	B500	118	201	9.3	3.15	2.5-17.5	42.7	8.88	19 -60.5
	B501	27	197	7.5	3.02	3.5-15	39.8	7.34	21 -54.5
	B504	42	232	9.0	2.80	3-15	37.1	8.77	18 -55.5
3	B499	22	-	9.6	2.71	4-12.5	-	-	-
	B503	89	-	9.0	2.63	2.5-14.5	-	-	-
	B505	135	-	11.3	2.96	4-17.5	-	-	-
	B510	58	-	9.9	2.65	4-16.5	-	-	-
4	B447	131	-	8.9	2.67	3-14.5	-	-	-
	B448	273	-	10.1	3.08	3-18	-	-	-
	B875	183	-	9.3	3.07	3-16.5	-	-	-
	B968	83	-	8.8	3.03	2.5-16	-	-	-

* The next smallest was $22\frac{1}{2}$ mm. long

There is no significant difference between the lengths of flukes of the same age in any of the groups, the variation between the animals within each group being

much larger than the variation between the groups.

No flukes were found in the small intestine of any calf, but they were recovered in very small numbers from the gall bladders of B500, B501, B504, B506, B509, B512 and B513. One fluke recovered from the liver parenchyma of B500 was 29½ mm. in length, 18 weeks after infection.

Other parasitic infections: Cysticercus bovis was found in B968 and some specimens appeared to be viable, while Dictyocaulus viviparus was found in B448. In both cases the infection was very light. Small numbers of paramphistomes were recovered from 12 of the animals.

3. Post-mortem examinations

Detailed post-mortem examinations were carried out on all the calves.

There were no signs of flukes in any of the control animals, nor any evidence that they had ever been so infected. In all other respects these animals also appeared entirely normal.

All the carcasses were in good condition and the normal amount of fat expected in animals of this breed was present. B498 and B501 each had a consolidated area of long-standing in one lung, and small organized fibrinous adhesions were seen on the pleura of some of the calves.

Full details of the liver lesions will be given in Chapter 15. There were no marked differences from the

lesions which are typical in infections of this type. However in B448, and to a lesser extent in B501, there were prominent areas of adhesions between the liver and surrounding organs.

Other post-mortem details are given in Table 13.5. Although there is considerable variation between the animals in each group, there is a tendency for the weights of the hepatic lymph nodes and of the liver and of the volume of the bile to increase with infection rate and time.

Table 13.5

Liver weights (mean and s.d.)

Group	Weight of liver (g.)	Weight of hepatic lymph nodes (g.)	Volume of bile (ml.)
1	5130±243	153±35	161±61
2	5700±670	187±78	220±182
3	4094±226	52±5	96±54
4	4508±263	61±17	98±51
C	4207±55	35±7	92±21

In every case the liver weight included the hepatic lymph nodes together with the full gall bladder.

4. Biochemical data

As it was not possible to carry out the biochemical tests on fresh sera, the sera were stored at -25°C; the results of the tests should be interpreted with this in mind.

(a) Iodine flocculation tests: The results of these tests, as carried out on sera stored for 7-10 months, are shown in Table 13.6.

Table 13.6

Iodine flocculation tests

Calf No.	Group	Weeks post-infection									
		2	4	6	8	9	11	12	14	16	18/19
B498	2	0	0	0	0	0	0	0	16	8	8
B500	2	0	0	0	0	0	0	0	8	6	6
B501	2	0	0	0	0	0	2	1	8	8	12
B504	2	0	0	0	2	12	4	8	8	4	12
B506	1	0	0	0	0	1	0	2	8	8	12
B509	1	0	0	0	0	0	4	0	8	12	12
B512	1	2	±	±	2	2	0	16	12	8	12
B513	1	0	0	0	0	0	0	4	2	8	0
B507	C	0	0	0	0	0	0	0	0	0	0
B511	C	0	0	0	0	0	0	0	0	0	0

± Indicates a doubtful result

The preinfection tests were all negative and the 2 minute reading was taken in every case as it was found to give more reliable results than the reading taken after 30 minutes. Each test was carried out with serum/reagent ratios of 1:1, 2:1 and 4:1 and the results are expressed as this ratio x reaction strength, scored 1-4.

Relatively consistent positive results were obtained in all infected animals by 9-14 weeks post-

infection. Occasional very early positive results would seem to indicate that a score of more than 4 is necessary to establish a positive result under these conditions. The challenge infection in Group 2 did not result in any distinct difference as compared with Group 1 which had no challenge infection.

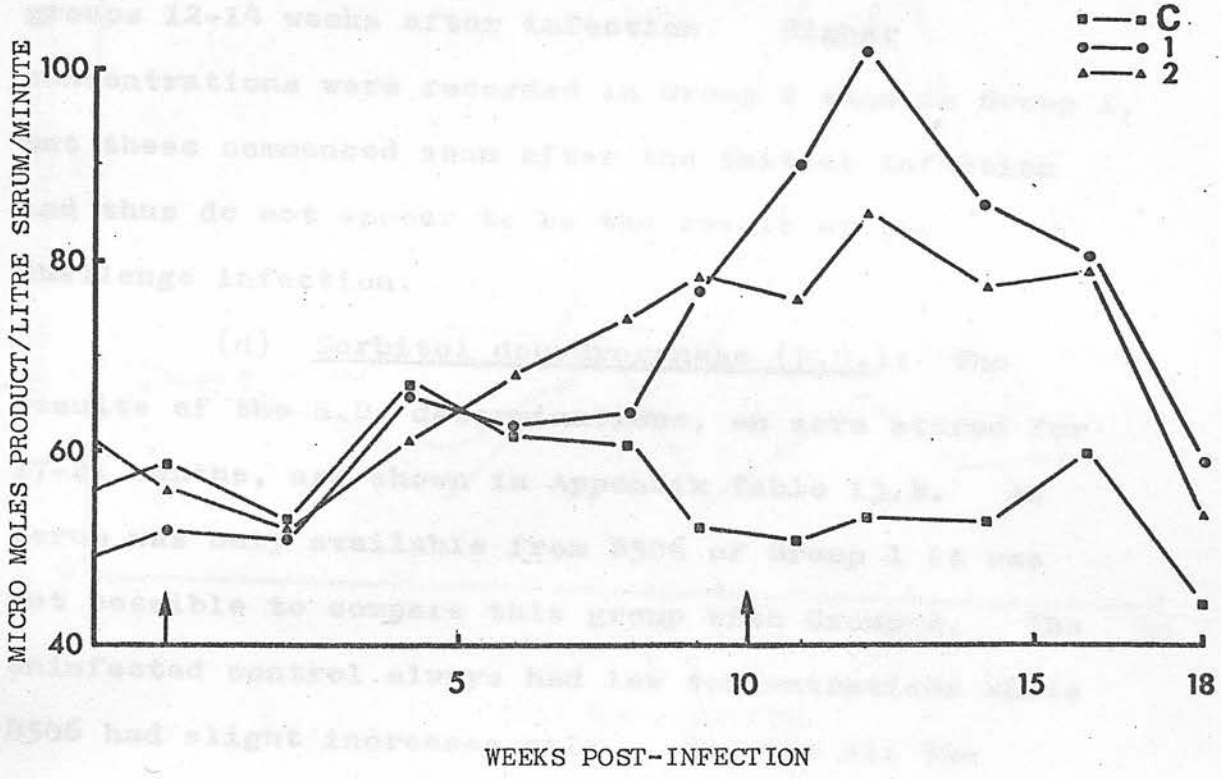
The results from Groups 3 and 4 were in line with those recorded previously from similarly infected animals except that the sera from B447 (Group 4) had a score of 12 from one week after infection until it was slaughtered. There were no changes in the serum protein fractions which might have accounted for this.

(b) Serum glutamic oxaloacetic transaminase (S.G.O.T.): The results of the S.G.O.T. determinations, on sera stored for 2-6 months, are shown in Appendix Table 13.B and Graph 13.2.

One of the uninfected controls (B511) had high concentrations on several occasions, while B498 of Group 2 showed only very slight and irregular increases. The graph indicates that the means of the concentrations of the infected groups were considerably higher than those of the uninfected controls, but that they were lower after the challenge infection in Group 2 than Group 1 which did not have a second infection.

The highest concentrations recorded were at 12-16 weeks in Group 2 and 12-14 weeks in Group 1.

(c) Ornithine carbamyl transferase (O.C.T.): The results of the O.C.T. determinations, on sera



GRAPH 13.2 SERUM GLUTAMIC OXALOACETIC TRANSAMINASE

stored for 7-12 months, are shown in Appendix Table 13.B and Graph 13.3.

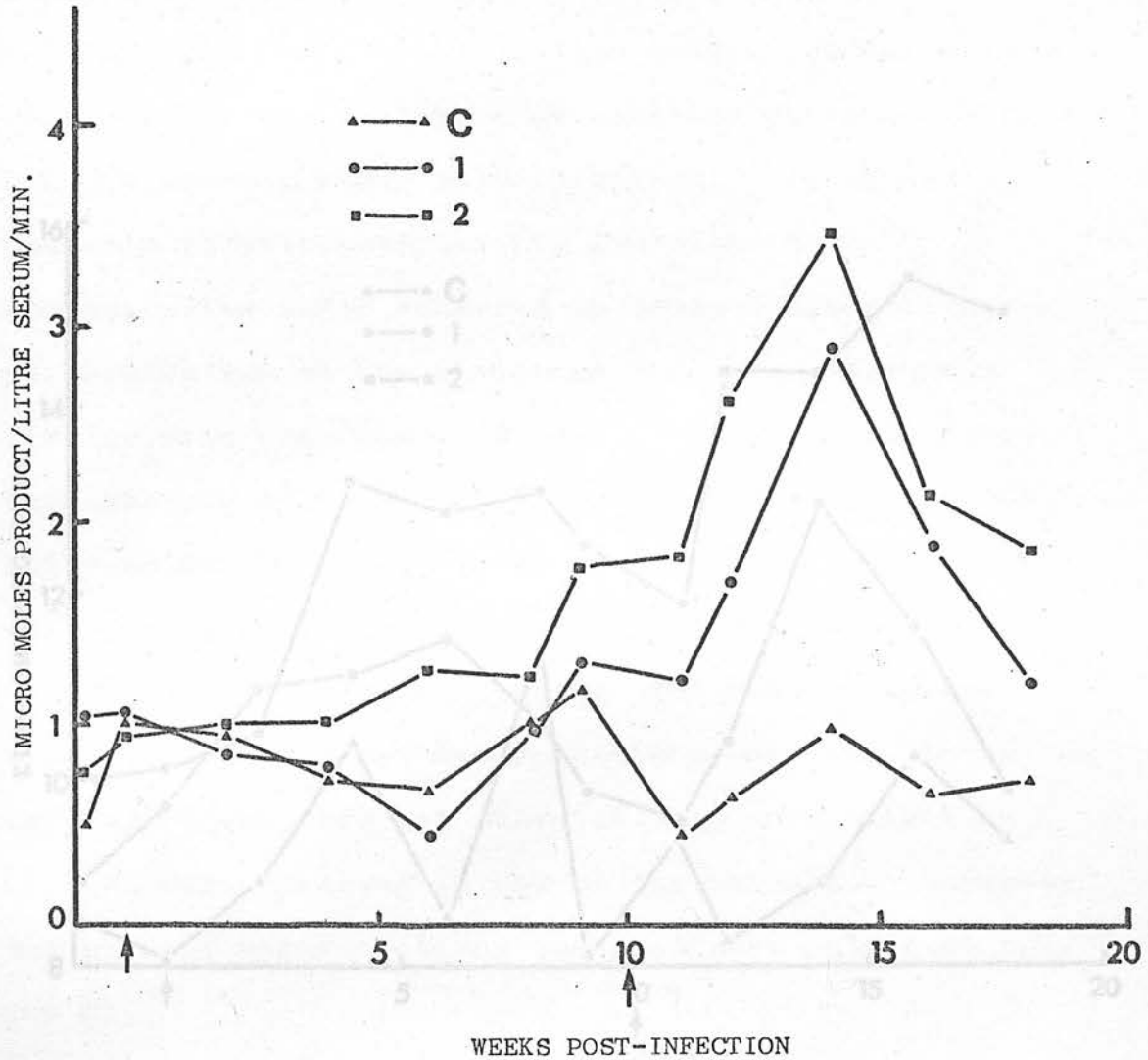
The concentrations in the uninfected control animals remained low, although there was a slight rise at 8 weeks after the time of infection in B511.

All the infected animals showed appreciable rises in concentration, peak levels being reached in both groups 12-14 weeks after infection. Higher concentrations were recorded in Group 2 than in Group 1, but these commenced soon after the initial infection and thus do not appear to be the result of the challenge infection.

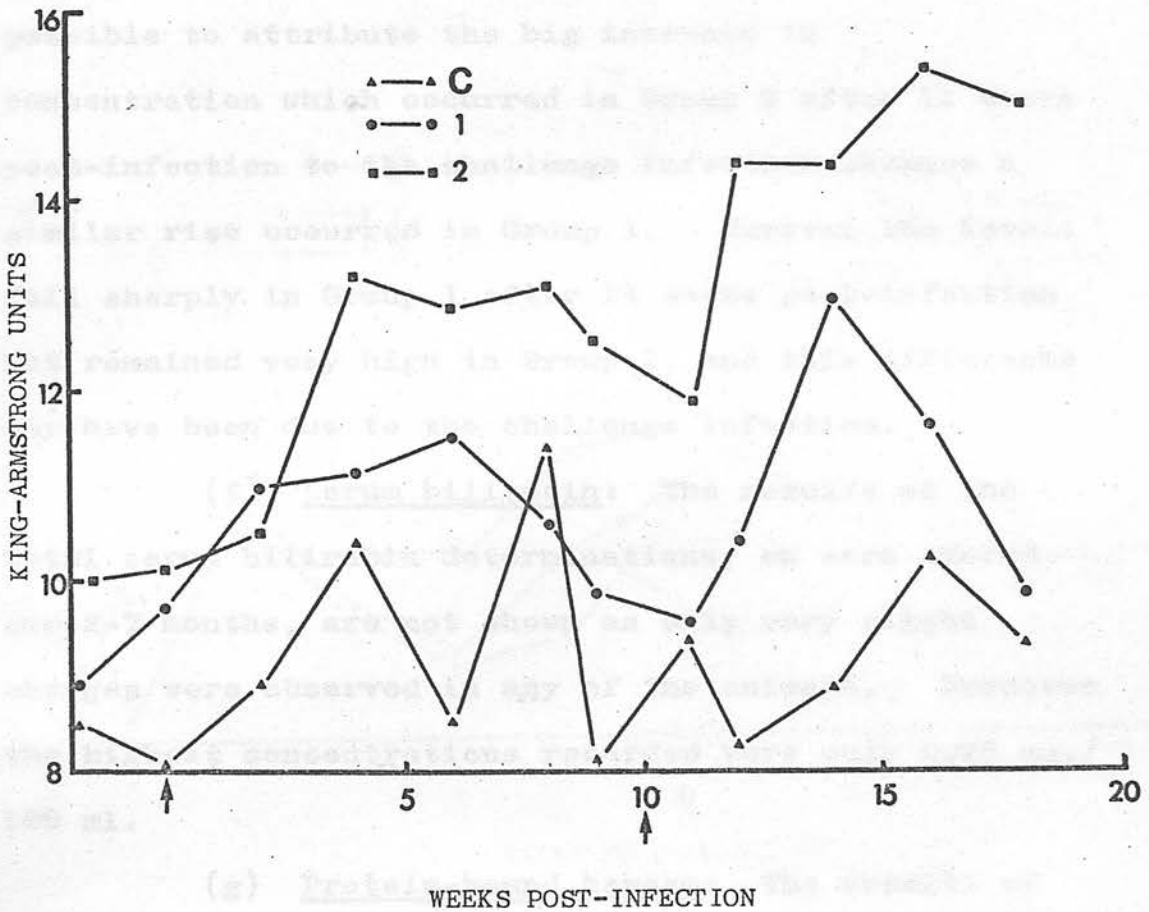
(d) Sorbitol dehydrogenase (S.D.): The results of the S.D. determinations, on sera stored for 17-21 months, are shown in Appendix Table 13.B. As serum was only available from B506 of Group 1 it was not possible to compare this group with Group 2. The uninfected control always had low concentrations while B506 had slight increases only. However all the animals in Group 2 showed large increases in concentration with peak levels from 13-17 weeks after infection.

GRAPH 13.3 ORNITHINE CARBAMYL TRANSFERASE

(e) Alkaline phosphatase: The results of the alkaline phosphatase determinations, on sera stored for 10-13 months, are shown in Appendix Table 13.B and on Graph 13.4. Compared with the concentrations in the control animals, which fluctuated about the initial levels, those in the two groups of infected



GRAPH 13,3 ORNITHINE CARBAMYL TRANSFERASE

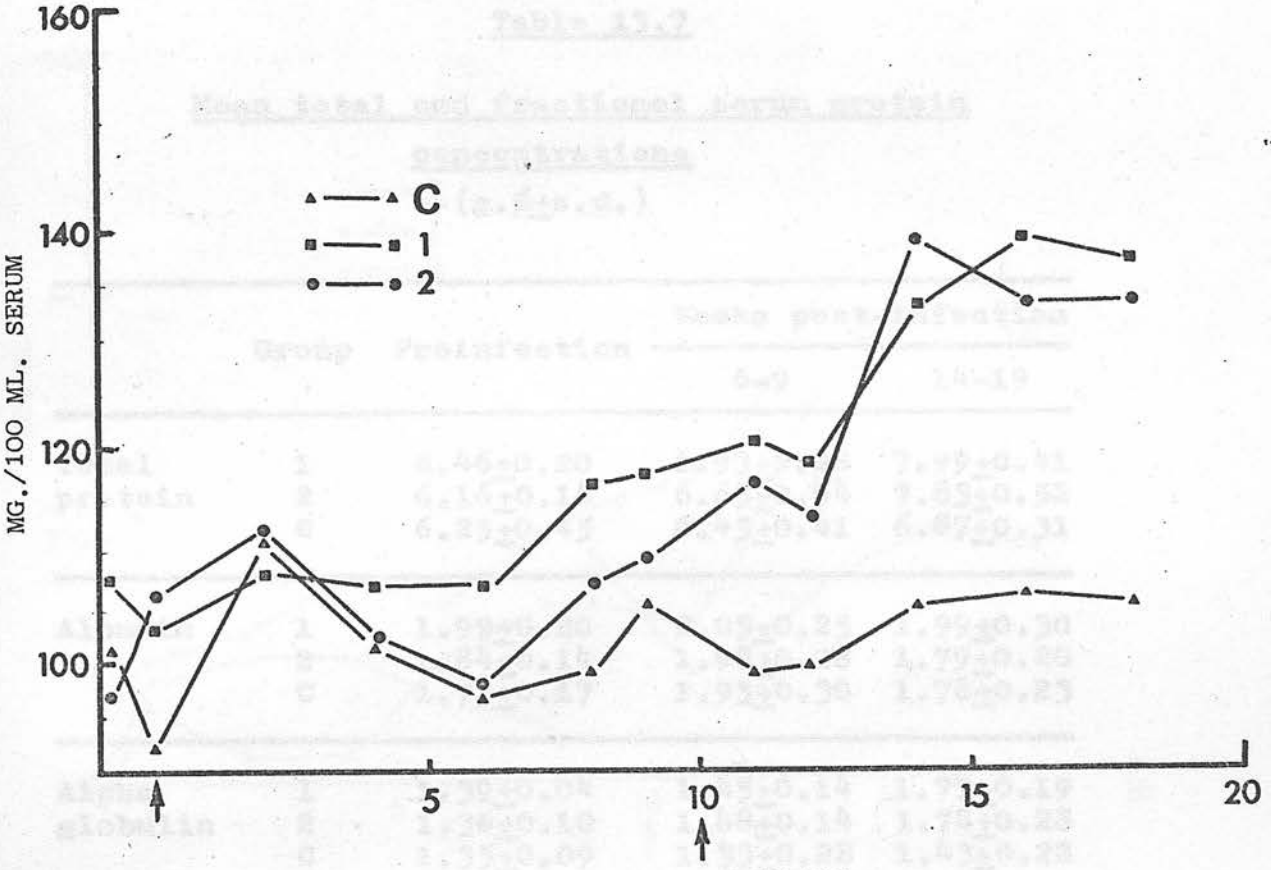


GRAPH 13.4 ALKALINE PHOSPHATASE

calves rose to two peaks, one at about 6 weeks and the other at 14-16 weeks after infection, with a well-defined fall at 11 weeks in each group. Group 2 had considerably higher levels of alkaline phosphatase than Group 1 after 2 weeks post-infection. It is not possible to attribute the big increase in concentration which occurred in Group 2 after 11 weeks post-infection to the challenge infection because a similar rise occurred in Group 1. However the levels fell sharply in Group 1 after 14 weeks post-infection but remained very high in Group 2, and this difference may have been due to the challenge infection.

(f) Serum bilirubin: The results of the total serum bilirubin determinations, on sera stored for 2-7 months, are not shown as only very slight changes were observed in any of the animals. Moreover the highest concentrations recorded were only 0.26 mg./ 100 ml.

(g) Protein-bound hexose: The results of these determinations, on sera stored for 3-6 months, are shown in Appendix Table 13.B and Graph 13.5. These tests were only carried out on two animals from each of Groups 1, 2 and C. While the initial levels were maintained in the uninfected controls, marked increases were seen in both the infected groups. The rate of increase was most marked from 12-14 weeks after infection, similar peak levels being reached at 14-16 weeks in both groups.



GRAPH 13.5 PROTEIN-BOUND HEXOSE

(h) Total serum protein concentration: The results of these assays, on sera stored for 9 months, are shown in Appendix Table 13.C, Table 13.7 and Graph 13.6.

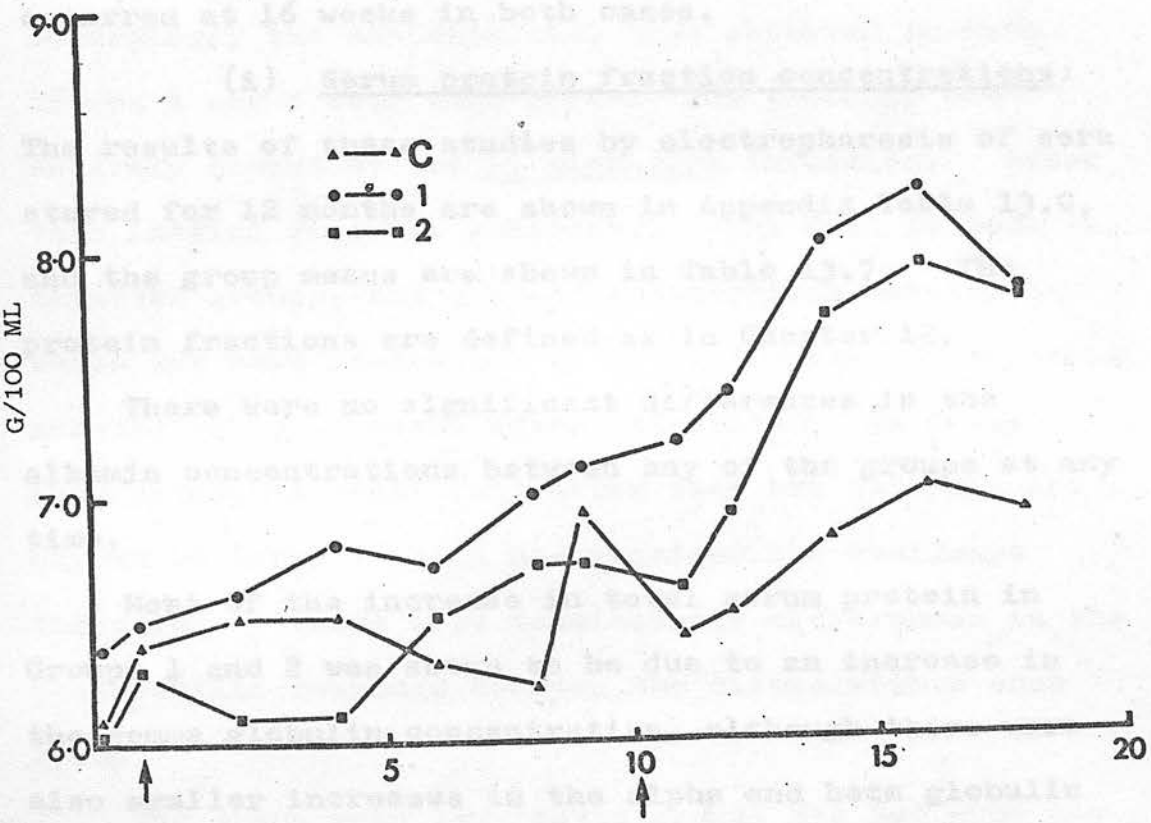
Table 13.7

Mean total and fractional serum protein concentrations

(g.%±s.d.)

	Group	Preinfection	Weeks post-infection	
			6-9	14-19
Total protein	1	6.46±0.20	6.93±0.28	7.99±0.41
	2	6.16±0.14	6.63±0.64	7.83±0.52
	C	6.25±0.45	6.45±0.41	6.87±0.31
Albumin	1	1.99±0.20	2.05±0.25	1.99±0.30
	2	1.84±0.14	1.98±0.28	1.79±0.20
	C	1.73±0.17	1.95±0.30	1.78±0.23
Alpha globulin	1	1.39±0.04	1.45±0.14	1.77±0.19
	2	1.36±0.10	1.48±0.14	1.78±0.28
	C	1.35±0.09	1.33±0.28	1.43±0.22
Beta globulin	1	0.80±0.13	0.89±0.10	1.06±0.10
	2	0.89±0.03	0.94±0.09	1.21±0.22
	C	0.83±0.05	0.88±0.13	0.97±0.13
Gamma globulin	1	2.33±0.20	2.52±0.23	3.17±0.31
	2	2.09±0.21	2.33±0.42	3.06±0.25
	C	2.35±0.26	2.43±0.32	2.67±0.20

There was a slight rise in the levels in the control animals over the experimental period but a much



GRAPH 13.6 TOTAL SERUM PROTEIN

greater rise in both the infected groups, although the levels were somewhat higher in Group 1 than in Group 2 throughout the experiment. The highest concentrations were reached in individual animals from 14 to 19 weeks after infection, while the highest group means occurred at 16 weeks in both cases.

(i) Serum protein fraction concentrations: The results of these studies by electrophoresis of sera stored for 12 months are shown in Appendix Table 13.C, and the group means are shown in Table 13.7. The protein fractions are defined as in Chapter 12.

There were no significant differences in the albumin concentrations between any of the groups at any time.

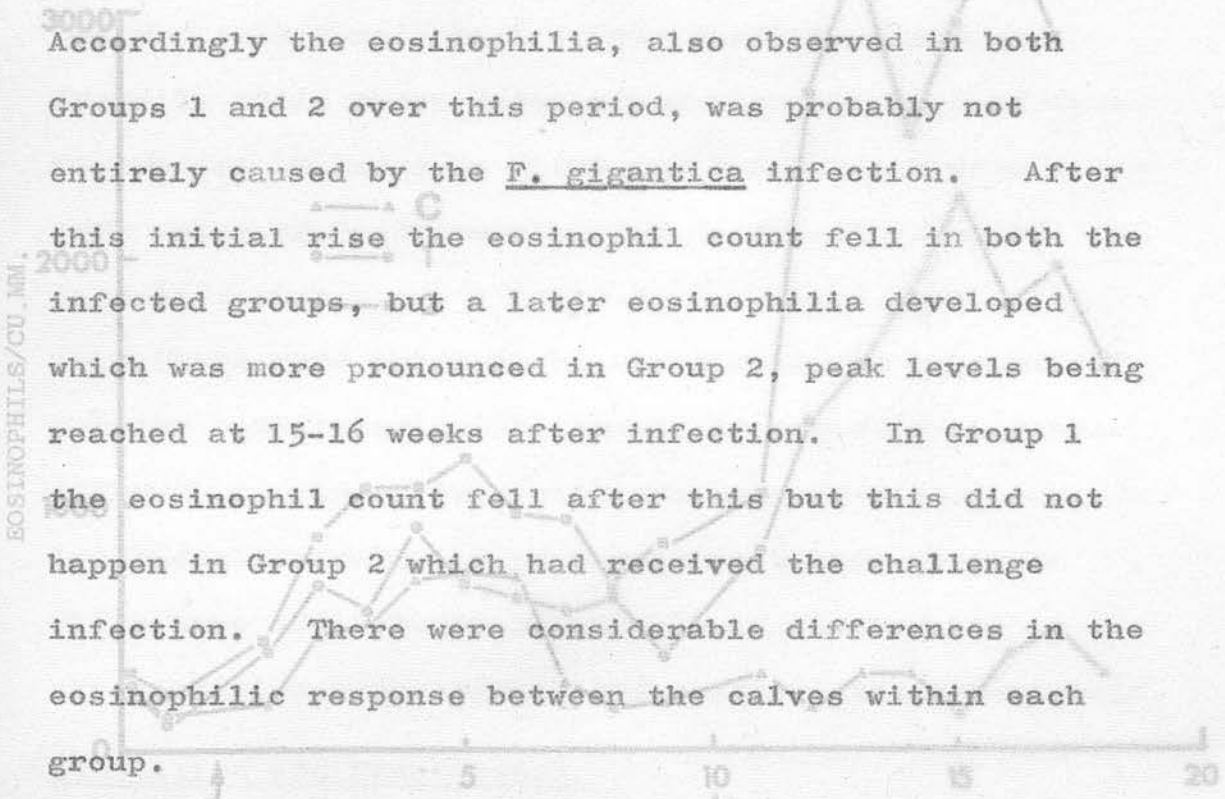
Most of the increase in total serum protein in Groups 1 and 2 was shown to be due to an increase in the gamma globulin concentration, although there were also smaller increases in the alpha and beta globulin fractions.

4. Haematological data

(a) The leucocyte series: Total and differential leucocyte counts are shown in Appendix Table 13.D. Interpretation of these counts over the period 6-12 weeks after infection is difficult because of the Eperythrozoon infections. However there was no distinct trend in the mean total leucocyte counts in either of the infected groups or the controls, over the whole experimental period, in spite of sometimes

considerable weekly variation.

The eosinophil counts are shown in Graph 13.7. There were consistently low counts in the control calves except during the period 2-6 weeks after the infection date when they developed a mild eosinophilia. Accordingly the eosinophilia, also observed in both Groups 1 and 2 over this period, was probably not entirely caused by the F. gigantica infection. After this initial rise the eosinophil count fell in both the infected groups, but a later eosinophilia developed which was more pronounced in Group 2, peak levels being reached at 15-16 weeks after infection. In Group 1 the eosinophil count fell after this but this did not happen in Group 2 which had received the challenge infection. There were considerable differences in the eosinophilic response between the calves within each group.

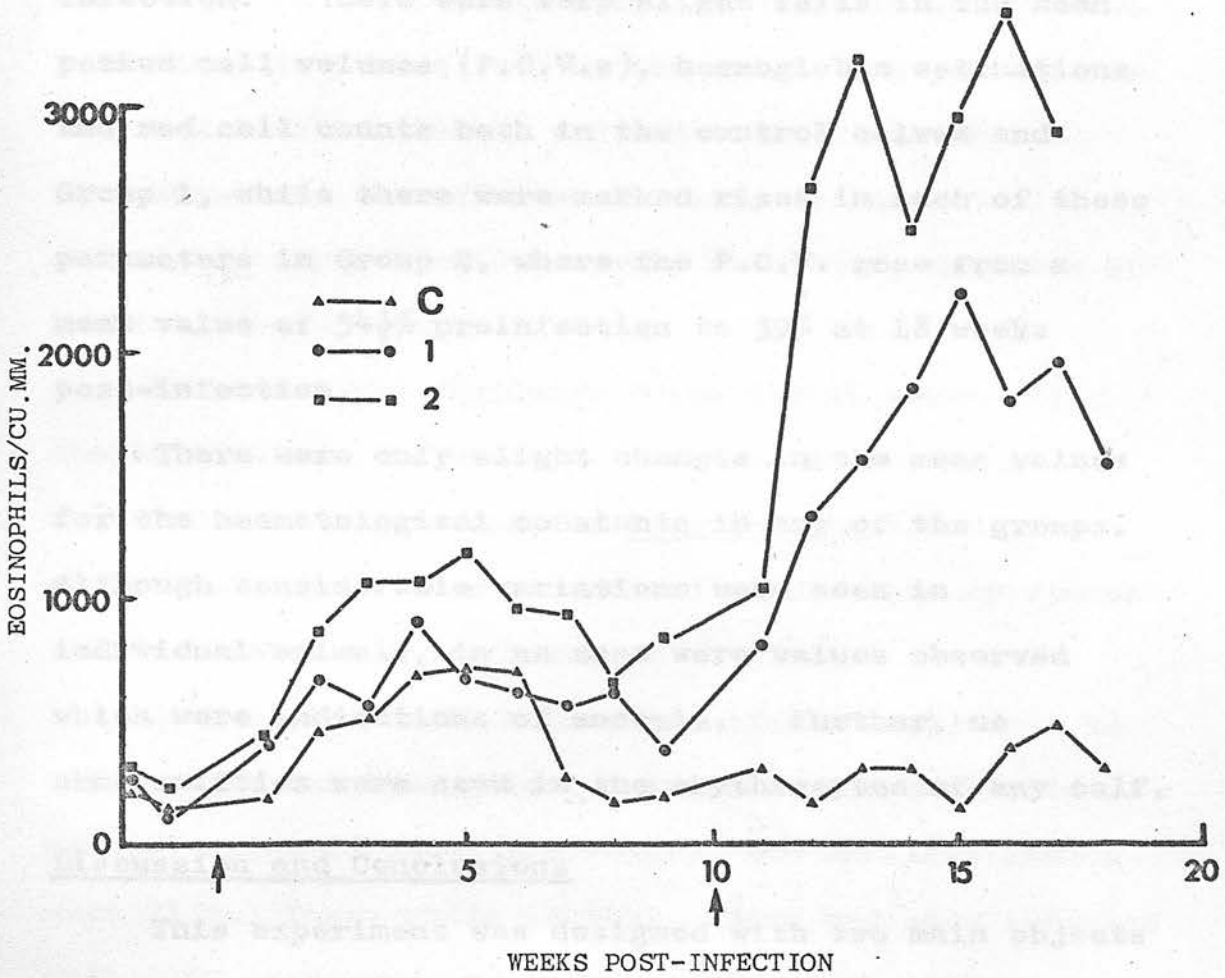


GRAPH 13.7 EOSINOPHIL COUNTS

The group mean lymphocyte counts did not show any definite trends although there were sometimes considerable weekly variations, and the same general picture applied to the neutrophils. Monocytes, basophils and band neutrophils fluctuated around the preinfection levels in all the groups throughout the experiment.

No toxic granulation or other indications of toxaemia were seen in the neutrophils of the infected calves.

(b) The erythrocyte series: The results are



GRAPH 13.7 EOSINOPHIL COUNTS

given in Appendix Table 13.E. Only B509 developed an anaemia during the course of the experiment. This was of short duration and associated with the Eperythrozoon infection. There were very slight falls in the mean packed cell volumes (P.C.V.s), haemoglobin estimations and red cell counts both in the control calves and Group 1, while there were marked rises in each of these parameters in Group 2, where the P.C.V. rose from a mean value of 34½% preinfection to 39% at 18 weeks post-infection.

There were only slight changes in the mean values for the haematological constants in any of the groups. Although considerable variations were seen in individual animals, in no case were values observed which were indications of anaemia. Further, no abnormalities were seen in the erythrocytes of any calf.

Discussion and Conclusions

This experiment was designed with two main objects in view. The first of these was to study the pathogenesis of a single infection of metacercariae at a lower dose level than previously, while the second was to study the effect of a challenge infection of the same size to see if it gave rise to any differences compared with the single infection, which might indicate a protective immune reaction.

The challenge infection was given 10 weeks post-infection, so that the young flukes would be entering the liver parenchyma at the time of maximal tissue

damage resulting from the initial infection, as suggested from the experiment described in Chapter 12. Further, as it had also been found that the flukes started to enter the bile ducts at about 10 weeks after infection, they were nearing the end of the period when they had been in closest contact with the liver parenchyma and therefore most likely to have stimulated any protective immune mechanism, cellular or humoral.

By giving the challenge infection 10 weeks after the initial one, it was also possible to see if an acquired self-cure occurs in F. gigantica infections under these conditions as the two populations of flukes could be readily distinguished.

It had previously been shown (Table 12.4) that in B456, which had been given an infective dose of 1000 metacercariae 16 weeks previously, the smallest flukes were 21 mm. long, while in B451, which had been infected with 1000 metacercariae 8 weeks before, the largest flukes were 12 mm. long. It was thought that it would therefore be possible to separate the two populations on size grounds, especially as any protective mechanism would probably tend to increase rather than decrease the difference in length between the populations of flukes. Therefore, while it was impossible to be certain of completely separating the two populations, it was thought that insufficient overlap would occur to affect the statistical analyses. In fact the two

populations were clearly separate and distinct in the animals in Group 2 (Table 13.4), there being less than 5 flukes in the intermediate range about 15-22 mm. long in any of the animals. It was therefore assumed that flukes over 17½ mm. long were from the initial infection, while smaller flukes were from the challenge infection.

It was also necessary to terminate the experiment before any adult flukes died and were lost from the liver. The only report in the literature on the longevity of F. gigantica is that of Alicata and Swanson (1941), who gave no details of infections of less than a year in length and merely indicated that a considerable reduction in numbers was likely 16 months after infection. However Ross (1968) had shown in cattle that with low level single infections of F. hepatica 75% were lost between 5 and 21 months after infection, and it was therefore considered advisable to terminate the experiment before 5 months after infection. Accordingly the animals were all slaughtered at 18 weeks after the experiment started when very few dead or degenerating flukes were seen. This will be referred to in more detail in Chapter 16.

The infective dose of 500 metacercariae which it was desired to use to study the pathogenesis of F. gigantica was also considered suitable for the study of the effect of the challenge infection, as about 300 flukes would be expected to develop from such an infection and this number might be large enough to

stimulate a protective immunity. Moreover such a level of infection was thought to be similar to those commonly seen in field cases. Therefore the two experiments could be linked together in one overall experiment.

Group 4 was included to allow for any possible "crowding effect", a single infection of 1000 metacercariae being compared with the two infections of 500 metacercariae in Group 2 to avoid simply comparing a smaller population, following a single infection, with a larger population, after single and challenge infections.

Group 4 was also used to provide additional information, both on the pathogenesis of early infections at this dose level and on fluke growth rates.

Group 3 was used primarily as a control for recovery rates and the lengths of the flukes of the challenge infection in Group 2, but also as a control for the pathogenesis of the infections to see if there was any difference in the effect of the challenge infection in Group 2. It was not desirable to use the single infection in Group 1 or the primary infection in Group 2 for the purpose of this comparison because of seasonal and age differences. Group 3 was also used to provide further information on the pathogenesis of early infections at this dose level and on fluke growth rates.

The statistically significant reduction in the

recovery of flukes in the challenge infection in Group 2, compared to the single infection in Group 3, may have been spurious because the young flukes were harder to recover from the much more fibrous parenchyma in the former. Further, if any retardation in growth had taken place, because the fibrosis had made the liver a less attractive environment for the fluke, a proportion would be below the size recovered by the technique used (Tables 12.2 and 14.3).

According to Neitz (1940) eperythrozoonosis in non-splenectomised cattle results in either a mild anaemia or none at all and this was the picture in these calves, where only one animal developed a mild anaemia out of the 7 known to have been infected with Eperythrozoon spp. However Neitz does not refer to the falls in leucocyte counts which were very marked in some cases, and which were always associated with the appearance of the parasite in the peripheral blood. Although only 7 calves were found to be infected with Eperythrozoon spp. it is considered likely that more may have become infected but were not detected because of the transitory parasitaemia.

The only parameter which showed a consistent and distinct difference between Group 2 after the challenge infection and Group 1, was the eosinophil count which showed a spectacular rise. Among the individual animals in Group 2 this rise in the eosinophil count was inversely proportional to the number of flukes

recovered from the challenge infection. This may indicate that a more intense cellular reaction had occurred which may have resulted in the elimination of some of the young flukes. It is less likely that this eosinophilia was the result of a big increase in hepatic cell destruction, because none of the other parameters which would have indicated this were consistently increased in comparison with Group 1; indeed the S.G.O.T. levels tended to fall (Graph 13.2).

The S.G.O.T. levels of one of the control calves, B511, were often high in comparison with the preinfection levels in all the other animals and the two other uninfected controls. It is difficult to account for this as all the other parameters were within the range found to be normal in this experiment. Furthermore, no explanation is readily available for the failure of S.G.O.T. levels in B498 of Group 2 to rise much above those found before infection when both O.C.T. and S.D. reached high levels. A further anomaly in this animal was that the highest S.G.O.T. concentration was recorded only 4 weeks after infection.

Of the two techniques used to determine the number of F. gigantica eggs in the faeces the sieving method resulted in a higher recovery rate, although both produced sufficiently comparable results to allow the same conclusions to be drawn.

The reduction in live-weight gain relative to the uninfected control calves and to Group 3, seen in

Groups 1 and 2 during the period 9-13 weeks post-infection, may indicate a period of adjustment by the host to the parasites which are entering the bile ducts at this time.

The loss of weight seen in Group 4, between 6 weeks after infection and slaughter at 8 weeks, may be associated with the presence of intercurrent *F. gigantica* and protozoal infections, even though there was no clinical breakdown of the premunities in any of the animals.

CHAPTER 14

Prolonged Chronic and Other Infections in Cattle (Fasciola gigantica)

Introduction

A series of small experiments was carried out with the aim of collecting more data on the gross pathology of Fasciola gigantica infections, and on the growth and longevity of this fluke in cattle. These fell into two groups, namely those which provided additional information over the first 10 weeks of the infection, and those which were concerned with chronic infections of more than 19 weeks duration.

Experimental design

(a) Animals

The 14 animals used in these experiments were either born at E.A.V.R.O. or were obtained from a farm with no recent history of fascioliasis. A817 was a female but the others were all castrated males.

All were Guernsey-type animals except A805 and A817 which were of the Ayrshire type, B378 which was an Ayrshire x zebu and 8329 which was of typical zebu conformation.

The animals were maintained as previously described.

(b) Treatments

These animals were infected as they became available and as the supply of metacercariae allowed.

At the time of infection B378 was $8\frac{1}{2}$ months old, B450 and B455 were 10 months old, B971 and B973 were 12 months old, A817, C27, C29, C32, C33 and C36 were 14 months old, A805 was 15 months old and C37 was 18 months old, while 8329 was 67 months old.

The infective dose of metacercariae given to each animal is shown in Table 14.1. Those used to infect C37 were a strain of ovine origin, as metacercariae of a strain of bovine origin were not available. In every case the metacercariae were less than 14 days old at the time of infection.

Table 14.1

Recovery of F. gigantica

Animal No.	Infective dose (metacercariae)	Experimental period (weeks)	No. recovered	Percent. recovery
C37	3,000	2	8	0.27
C29	2,000	3	1	0.05
C27	2,000	4	240	12.0
C33	1,000	5	283	28.3
C36	1,000	6	464	46.4
C32	1,000	10	559	55.9
B450	500	20	321	64.2
B455	500	24	276	55.2
B378	160	26	89	55.6
8329	160	26	59	36.9
A805	700	30	387	55.3
B971	400	37	170	42.5
A817	600	43	97	16.2
B973	400	51	74	18.5

(c) Observations

Red cell counts, haemoglobin estimations and packed cell volumes were determined at the time of slaughter,

in the animals which had been infected for more than 19 weeks.

In most cases the prepatent period for F. gigantica was found. Egg counts were carried out at irregular intervals.

The flukes were recovered and all the undamaged ones measured.

Results

1. Clinical data

No symptoms were seen in any of the animals which could be attributed to fascioliasis and they were all in good bodily condition at slaughter. Prior to the infection with F. gigantica B455, B971 and B973 reacted positively to the capillary agglutination test for Babesia bigemina infection, and B450 to that for Anaplasma marginale, but in neither of these animals were clinical symptoms of these diseases seen during the experiment.

2. Parasitological data

The prepatent periods for F. gigantica, as recorded in the animals infected for more than 19 weeks, are shown in Table 14.2, together with the F. gigantica egg counts from the faeces and gall bladders. C32 had no F. gigantica eggs in the gall bladder or faeces when slaughtered 10 weeks after infection. The egg counts were carried out using the sedimentation technique except in the case of A805 and

Table 14.2

F. gigantica egg counts

Animal No.	Prepatent period (days)	E.p.g. at weeks post-infection																			No. of eggs in gall bladder	No. flukes recovered
		18	19	20	21	22	23	24	25	26	28	30	32	37	39	40	42	43	45	49		
B450	87	42	-	119																	19,300	321
B455	88	26	-	-	-	-	25	33													105,800	276
B378	92	14	-	19	16	16	33	7	10	11											18,000	89
8329	94	-	-	1	5	2	6	1	7	4											12,500	59
A805	-	-	-	-	-	-	-	-	-	-	6	9									37,200	387
B971	90	-	5	-	-	-	35	-	-	-	13	-	5	5							19,900	170
A817	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	1	1	1			13,800	97
B973	99	-	8	-	-	-	7	-	-	-	10	-	6	2	-	3	-	-	1	1	7,000	74

A817 where the sieving method was used.

The F. gigantica egg counts were uniformly low in these chronic infections and were very low in those animals which had been infected for 40 weeks or more. Even A805, from which 387 flukes were recovered at slaughter 30 weeks after infection, gave counts of less than 10 e.p.g. of faeces during the three previous weeks.

The two animals which were slaughtered more than 39 weeks after infection had significantly lower percentage recoveries of flukes than those slaughtered at any time from 6 weeks post-infection. Furthermore B971 and B973 each contained 4 degenerating flukes and A817 had 8 such flukes.

Details of the growth and size of F. gigantica in these animals are shown in Table 14.3.

There were 17 flukes in the small intestine of B378, 134 in the gall bladder of B455 and 93 in the gall bladder of B971. In both these latter animals the gall bladder wall was $\frac{3}{4}$-1 cm. thick and the mucous membrane contained calcium deposits. The empty gall bladder of B971 weighed 124 gm. The gall bladder wall of A817, in which 8 flukes were found, was also thickened with some areas of calcification.

Three flukes were recovered from the liver parenchyma of B973 when it was slaughtered 51 weeks after infection, these being $35\frac{1}{2}$, $34\frac{1}{2}$ and $26\frac{1}{2}$ mm. long. These flukes were still not mature as there were no

eggs present in their uteri.

Table 14.3

Growth of F. gigantica (length in mm.)

Animal No.	Experimental period (weeks)	Area of liver	No. measured	Mean	S.D.	Range
C37	2	-	7	1.1	0.19	1-1.5
C29	3	-	-	-	-	-
C27	4	-	19	3.4	0.70	2-4.5
C33	5	-	121	3.5	1.12	1-6
C36	6	1	34	5.8	1.55	2.5-8.5
		2	24	5.5	1.93	1.5-9.5
		3	33	6.0	1.39	3.5-9.5
		Total	91	5.8	1.60	1.5-9.5
C32	10	Unknown	22	20.6	6.36	6.5-29.5
		1	59	18.4	5.46	4-27.5
		2	48	19.4	5.59	7.5-30
		3	18	13.8	4.31	6-22
		Total	147	18.5	5.79	4-30
B450	20	-	281	45.4	7.05	26-58
B455	24	-	249	41.1	5.90	25-56
B378	26	-	67	45.8	4.40	29-54
8329	26	-	38	46.5	8.55	25-58
A805	30	-	238	30.5	5.88	12-45
B971	37	-	130	38.5	4.73	22-45.5
A817	43	-	48	37.6	5.11	26-50
B973	51	-	44	35.0	6.09	25-51.5

Other parasitic infections: Paramphistomes were recovered from C27, C29, C33, C36, C37, B378, 8329, B971 and B973, while Cysticercus bovis was found in C29, C36, B455 and B973, being apparently viable in C36 and B973. Two specimens of Dictyocaulus viviparus were also recovered from B971.

3. Post-mortem examinations

Detailed post-mortem examinations were carried out

on all animals. The liver weights and other details are given in Table 14.4.

Table 14.4

Some experimental details

Animal No.	Experimental period (weeks)	No. flukes recovered	Weight of liver (g.)	Weight of hepatic lymph nodes (g.)	Vol. of bile (ml.)	Live-weight (lbs.)
C37	2	8	6171	43	173	693
C29	3	1	4540	-	-	-
C27	4	240	4330	29	130	-
C33	5	283	4800	27	-	-
C36	6	464	3082*	-	-	-
C32	10	559	4342	87	125	582
B450	20	321	6155	195	64	519
B455	24	276	6520	180	160	532
B378	26	89	4710	-	122	520
8329	26	59	8455	109	162	-
A805	30	387	7204	185	415	813
B971	37	170	7763	135	94	663
A817	43	97	9855	170	330	591
B973	51	74	9334	160	240	880

* In this animal the liver weight does not include the hepatic lymph nodes or the gall bladder.

C28, details of which are given in Chapter 13, affords an uninfected control for C32, B450, B455 and B378, as it was the same age and was kept in the same way as these animals. However, as these 5 animals were killed over a period of 4 months, there were grazing and seasonal differences which preclude a strict comparison of live-weights.

No controls were kept for the other animals. It

would appear that the liver weights of the two animals which lived the longest are much higher than if merely related to the size of these older animals.

Full details of liver lesions are given in Chapter 15. There were no marked differences from the lesions described there, except in the case of C36 which had 3 small encapsulated abscesses in the liver (Plate 15.5).

Small organized fibrinous adhesions were seen on the pleura of most of the animals, while two abscesses, which contained inspissated pus, were found in the lungs of B973.

The carcasses of all the animals were in good condition and contained the normal amount of fat expected in animals of these breeds, under this system of management.

4. Haematological data

The results of the haematological examinations are given in Table 14.5.

Preinfection determinations were not made but it can be seen that anaemia did not develop in any of these animals. The packed cell volumes of both B450 and B455 were 30½% at 8 weeks after infection, so that this parameter rose between this time and when these animals were slaughtered 12 and 16 weeks later respectively. The mean cell volume (M.C.V.) of B455 was also determined 8 weeks after infection when it was found to be 52.6 cu.μ., very little different from that

Table 14.5

Terminal haematological results

Animal No.	Experimental period (weeks)	No. flukes recovered	Red cells (million/cmm.)	Haemoglobin (g./100 ml.)	Packed cell volume (%)	Mean corpuscular volume (cuμ)	Mean corpuscular haemoglobin concentration (%)
B450	20	321	-	10.8	34	-	31.3
B455	24	276	6.23	10.0	32	51.6	31.3
B378	26	89	-	-	46½	-	-
8329	26	59	-	-	50½*	-	-
A805	30	387	8.59	12.9	41	47.9	31.4
B971	37	170	9.43	11.9	39	41.5	30.6
A817	43	97	7.40	13.0	38½	52.0	33.8
B973	51	74	6.65	11.7	36	54.1	32.4

* 8329 was very wild when the blood sample was taken

found 24 weeks after infection. Although the M.C.V.s of most of the animals are slightly above those given by Schalm (1965) and Holman (1956) for animals of the same age, they are of the same order as those in the uninfected control, C28, (Chapter 13) or in the preinfection determinations in the associated animals in Group 4 (Chapter 13).

Discussion and conclusions

Most of the findings in these animals will be compared with those in the other experiments and with other published work in the general discussion in Chapter 16.

However there was one observation which only occurred in the prolonged infections, this being the large numbers of flukes in the gall bladders of B455, B971 and in smaller numbers in the same site of A817. It seems likely from the lesions that flukes had been living in these gall bladders for some considerable time. No definite reason can be given as to why they had moved from the bile ducts to the gall bladder, where they are rarely found to be living, especially in the numbers recorded in B455 and B971. This may be associated with the fibrotic bile ducts becoming an unsuitable environment for the flukes, but if so a similar response did not occur in the other animals.

CHAPTER 15

The Gross Pathology of Fasciola gigantica Infections in Cattle

Introduction

Few studies of the pathology of *Fasciola gigantica* in cattle have been reported. Sewell (1962) in Nigeria described the lesions in experimentally infected cattle. These were mature infections except for one animal. Guralp, Ozcan and Simms (1964) briefly described the gross pathology in experimentally infected cattle in Turkey, while Coyle (1958, 1961) referred to the lesions in natural infections in Uganda. Finally Bitakaramire (1969) and Bitakaramire and Bwangamoi (1969) described the lesions in experimentally infected calves in Kenya.

Observations were therefore made of the gross pathology in the experimentally infected animals used in the studies described in Chapters 12-14. These observations were then correlated with the results of the biochemical and haematological studies on these same animals.

Results

The liver weight, together with the weight of the hepatic lymph nodes and the volume of bile, of most of the animals used for these observations are recorded in Tables 12.2, 13.5 and 14.4. The appearance of the bile did not differ materially from that recorded in

similar infections with Fasciola hepatica.

The description of the development of the lesions may be conveniently considered under three headings:-

1. The acute phase, 2-12 weeks after infection.
2. The chronic phase,
   (a) early chronic infections, 15-20 weeks after infection,
   (b) late chronic infections, 24-51 weeks after infection.
3. Challenge infections
   (a) long interval,
   (b) short interval.

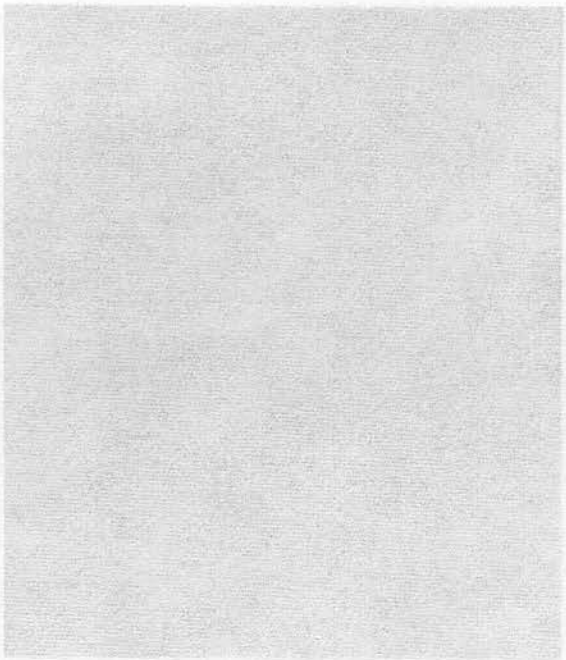
1. The acute phase

Two week infection - C37 (3000 metacercariae).

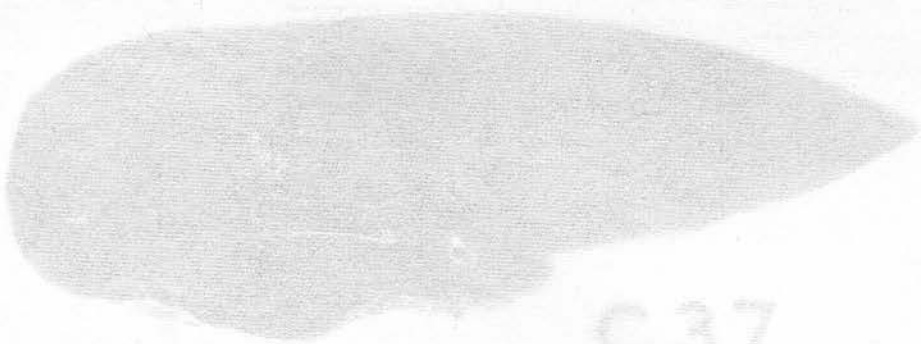
Numerous greyish-white subcapsular irregular lines 1-3 mm. long and up to $\frac{3}{4}$ mm. diameter were prominent on the surface of the liver. Plate 15.1 shows such tracks. These lesions were raised slightly above the surface of the liver and were more numerous in the ventral lobe. The tracks were sometimes confluent and in these cases they were usually surrounded by a thin red zone. On the cut surface of the liver the lesions had grey-yellow contents and were also surrounded by a thin red zone. The flukes had reached the centre of the liver by this time and the distribution of the tracks can be seen in Plate 15.2.

Three week infection - C29 (2000 metacercariae).

Fluke tracks were prominent on the surface of the



Plates 15.1 and 15.2. Liver lesions from C37 after a two week infection with 3000 metacercariae. (millimetre scale).




C 37


C37

liver and were again more numerous in the ventral lobe. The lesions were greyish in colour and were slightly raised above the surface of the liver. They were ¾-1 mm. wide, tortuous and occasionally up to 2 cm. long although some were very short. When closely grouped together they formed irregular mottled areas such as can be seen in Plate 15.3. Similar but less clearly defined tracks were seen on the cut surface of the liver.

No congested zone was seen round any of the lesions which were filled with a pale greenish material. The liver was not obviously congested.

Four week infections -

(a) C27 (2000 metacercariae) - The fluke lesions were much less conspicuous than in C29, because they were darker in colour and the liver was also slightly congested. The larger tracks were about 1 mm. wide and were deep yellowish-red with reddish borders, but the smaller ones were uniformly dark red. They were slightly raised above the surface of the liver and contained pale greenish material.

(b) B454 (1000 metacercariae) - The lesions did not differ materially from those seen in C27 except that they were fewer in number and even less conspicuous on the liver surface.

Five week infections -

(a) C33 (1000 metacercariae) - The fluke tracks immediately under the liver capsule were as well


C29


C 33

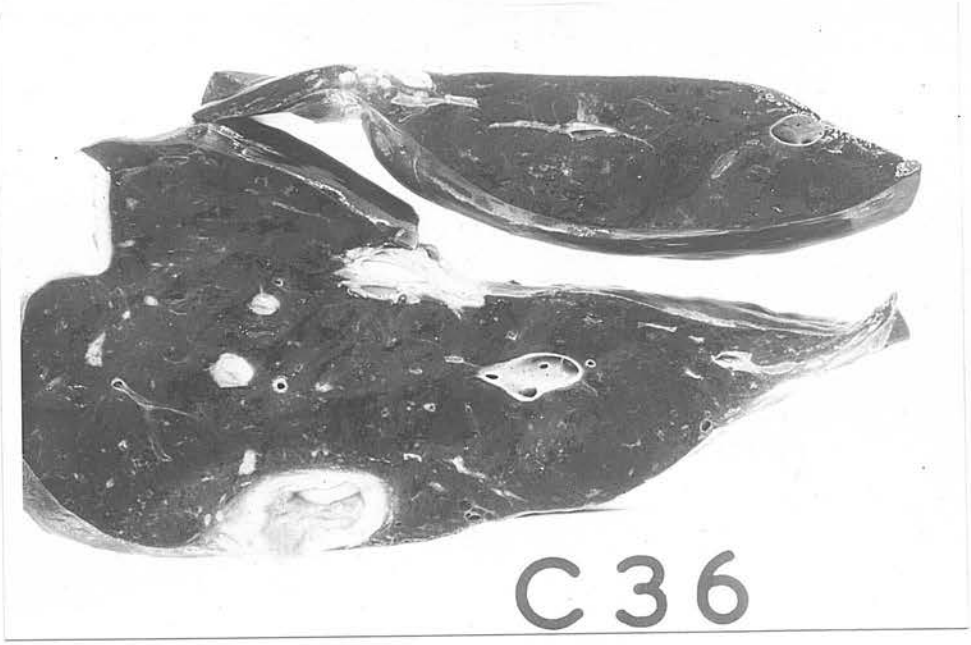
defined as in C27. The general appearance of these surface lesions is shown in Plate 15.4. They were slightly raised above the surface of the liver and were 1-1½ mm. across. The tracks contained light brown material and were surrounded by a wider zone of reaction than in C27.

(b) B460 (2000 metacercariae) - The picture here was very similar to that seen in C33 except that many more tracks were seen.

Six week infection - C36 (1000 metacercariae).

This liver was largely covered by fibrinous surface adhesions and was also slightly congested. The fluke tracks immediately under the liver capsule were dark in colour and poorly differentiated but they were clearly seen on the cut surface. These lesions appeared less numerous in area 3 (ventral) than elsewhere. However the flukes were least concentrated in area 2 (central) (Table 12.3). The distribution of the lesions is shown in Plates 15.5 and 15.6. Plate 15.5 also shows an abscess on the lower edge of the larger of the two slices of liver as well as two thickened bile ducts (area 2, central). One thickened bile duct can be seen in the smaller slice of liver (area 3, ventral). These thickened bile ducts contained pus.

The fluke tracks were slightly raised above the surface of the liver, they were 1-1½ mm. in diameter and most were light red-brown surrounded by a red zone,


C 3 6


C 3 6

but in some there was no such apparent peripheral reaction. Plate 15.7 shows such fluke tracks with little tissue reaction present. The contents of the tracks were mostly pale green-brown and were firm, but in some the contents were more red although none were haemorrhagic. Some tracks appeared to be empty. In general the tissue reaction was not well marked.

There was a tendency for the fluke tracks to become localized. The flukes appeared to burrow around a fixed point, and one such circumscribed lesion can be seen in Plate 15.7. These lesions were up to 1 cm. in diameter but were few in number at this stage.

Eight week infections -

(a) B499, B503, B505 and B510 (500 metacercariae) - The fluke tracks were not prominent on the surface of the liver and were hard to distinguish from the parenchyma. No part of the liver appeared to be more fibrotic than in the uninfected control calves. The fluke tracks were of two types. Those apparently more recently formed were 1-2 mm. in diameter and dark red in colour while others, which appeared to be older, were paler and narrower. The more recent lesions are shown in Plate 15.8. There was only a little more tendency for the fluke tracks to become restricted to circumscribed areas than in the 6 week old infection.

(b) B447, B448, B451, B875 and B968 (1000 metacercariae) - The fluke tracks were smaller than in those livers with the lower infection and were only

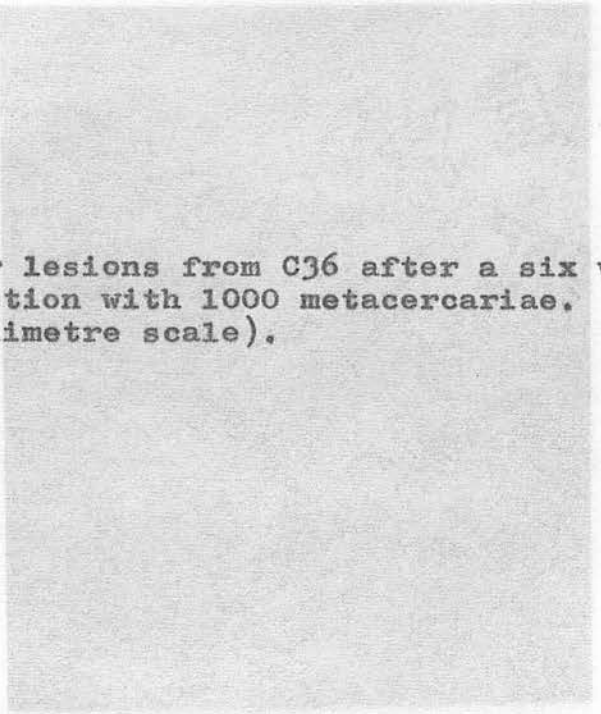


Plate 15.7. Liver lesions from C36 after a six week infection with 1000 metacercariae. (millimetre scale).

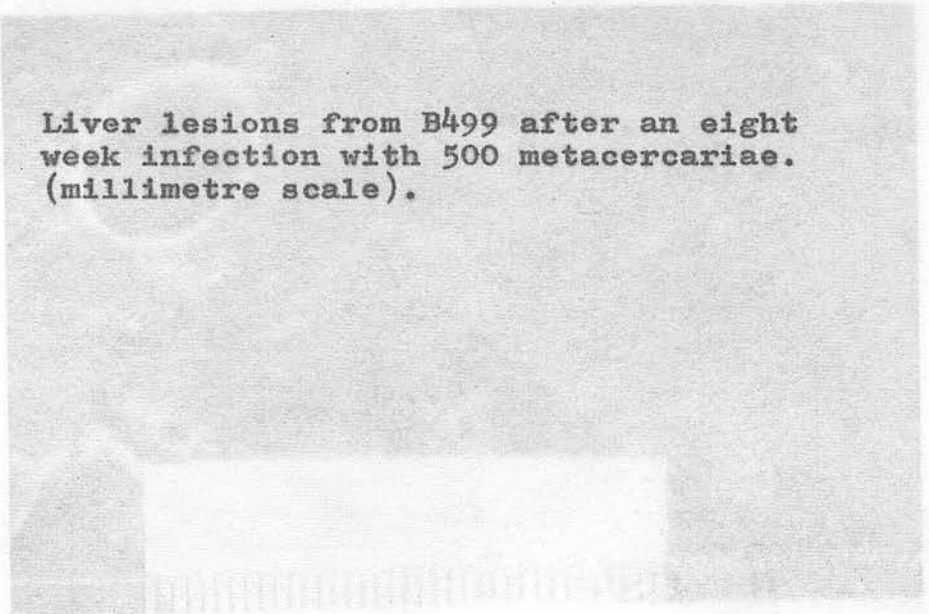


Plate 15.8. Liver lesions from B499 after an eight week infection with 500 metacercariae. (millimetre scale).


C 36


B 199

about 1-1½ mm. in diameter. There was also less tendency for the tracks to form circumscribed lesions. The liver of B447 appeared to be slightly more fibrotic than any of the others which appeared normal in this respect. Otherwise the lesions were as described for the former calves.

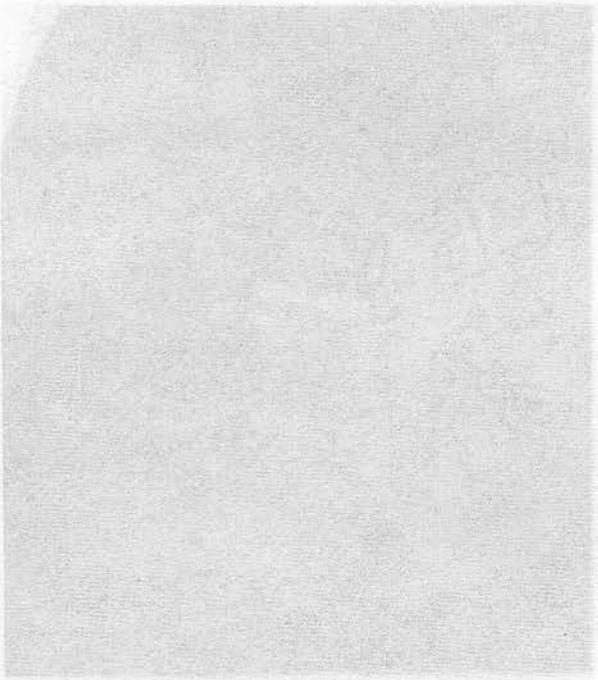
(c) B452 (2000 metacercariae) - The lesions were much the same as in the previous group, except that the ventral lobe appeared to be slightly fibrotic while the rest of the liver was normal in texture.

No flukes were found in the bile ducts in any of the 8 week old infections.

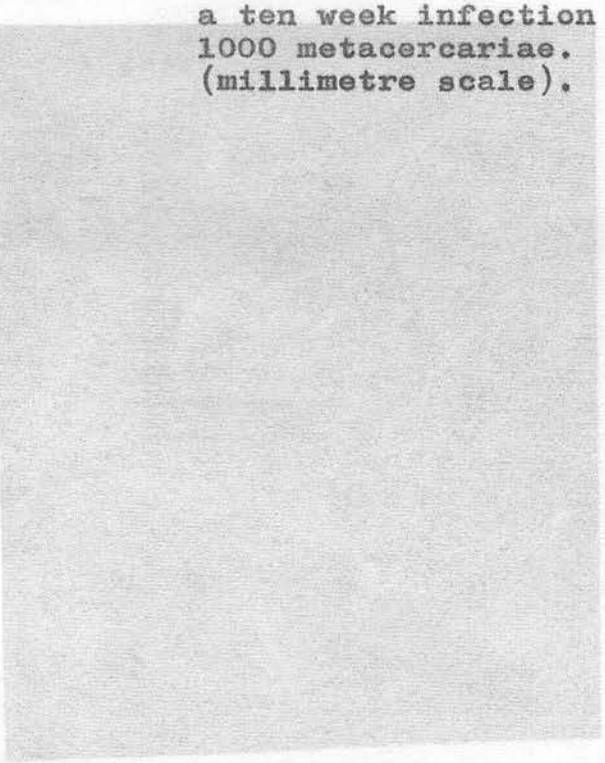
Ten week infection - C32 (1000 metacercariae).

The lesions were not clearly seen on the surface of the liver but were very marked on the cut surface. The fluke tracks were 1-2 mm. in diameter and the contents were usually yellow-brown to reddish-brown, but some were filled with what appeared to be clotted blood. The circumscribed lesions were more numerous than in the 8 week infections and were up to 1 x 1 cm. in diameter. Liver damage seemed more diffuse than at any other time. Plates 15.9 and 15.10 show these lesions.

There was some fibrosis throughout the liver, especially in the ventral lobe. A few flukes were found in the bile ducts, but the walls were not noticeably thickened.



Plates 15.9 and 15.10. Liver lesions from C32 after a ten week infection with 1000 metacercariae. (millimetre scale).




C 32


C 32

Twelve week infections -

(a) B458 (1000 metacercariae) - The fluke tracks were haemorrhagic and mostly confined to circumscribed areas, which had not yet become fibrous. These lesions could also be seen indistinctly under the liver capsule. In several cases flukes had penetrated the capsule and bleeding had occurred into the peritoneal cavity. A lesion of this type from another animal is shown in Plate 15.11.

Many flukes had already left the parenchyma and the walls of some of the bile ducts were becoming thickened. Parenchymal fibrosis was becoming marked especially in the ventral lobe.

(b) B449 (2000 metacercariae) - This liver closely resembled that of B458 although fewer flukes had penetrated the liver capsule. However the infection seemed to be less developed than in B458.

In both these livers the gross pathology was not of the diffuse type, which was seen in C32 at 10 weeks, but of the focal type seen in the earlier infections. This is shown in Plate 15.12. In infections of 12 weeks and over, recent fluke tracks were always haemorrhagic in nature.

2. The chronic phase

(a) Early chronic infections:

Fifteen week infection - B502 (2000 metacercariae) - This liver was orange-brown but this

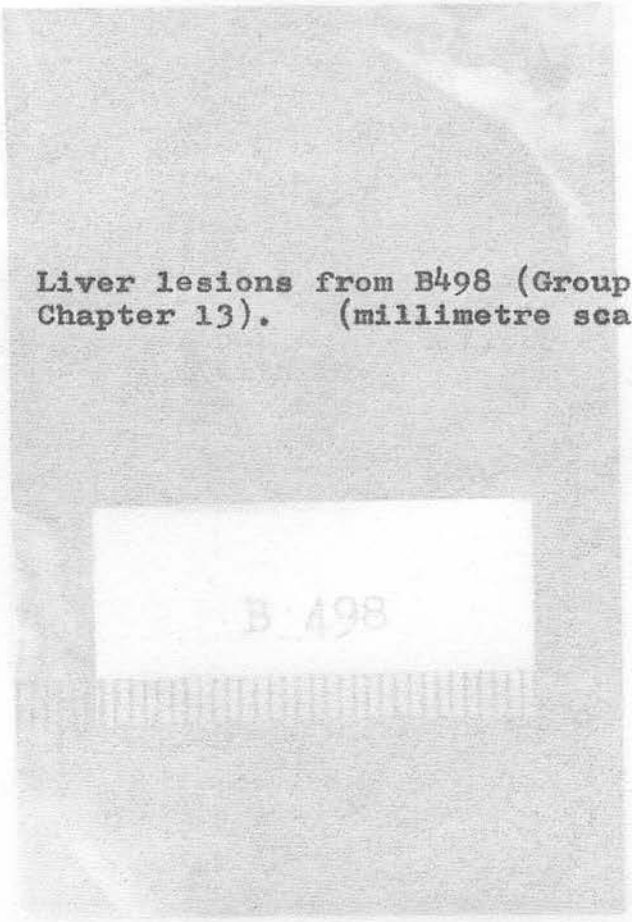


Plate 15.11. Liver lesions from B498 (Group 2, Chapter 13). (millimetre scale).

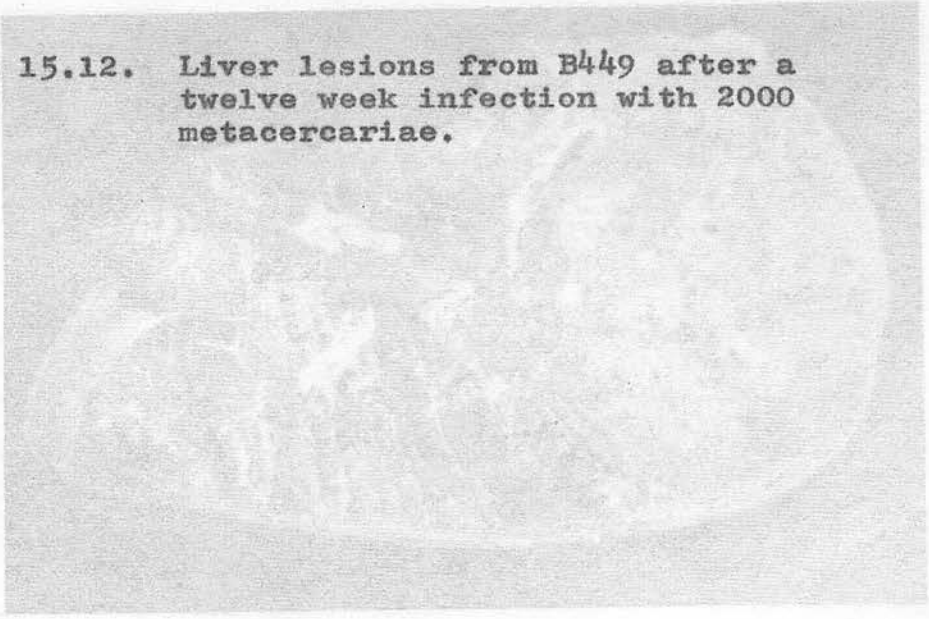


Plate 15.12. Liver lesions from B449 after a twelve week infection with 2000 metacercariae.


B 498
9
10
11
12

may have been due to toxic changes caused by the 'Spirotrypan forte' (Hoechst) which was given 3 days before slaughter. The bile ducts were noticeably more thickened and the fluke tracks fewer in number than in the calves with 12 week infections, as can be seen in Plate 15.13. Most of the fluke tracks were in circumscribed areas of two main types, one of which was more fibrous and therefore probably older than the other. A few flukes had penetrated the liver capsule.

The whole liver was more fibrous than in the 12 week infections, especially the ventral lobe. Very small areas of calcification of the bile duct were confirmed histologically, this being the earliest infection in which this was seen.

A prominent feature in this liver was localized dilatations of the bile ducts. These were up to 6 cm. long and were filled with flukes and a dark-coloured viscous fluid which appeared to contain blood.

Sixteen week infection - B456 (1000 metacercariae). The bile duct walls were much thicker and calcification was more evident than in the 15 week infection. There were also fewer flukes in the parenchyma and the more fibrous type of circumscribed lesions were more numerous than the others. These fibrous circumscribed lesions were about 1 x $1\frac{1}{2}$ x 1 cm. in size and are perhaps best described as fibrous "nodes". These lesions are shown in Plates 15.14 and 15.15.

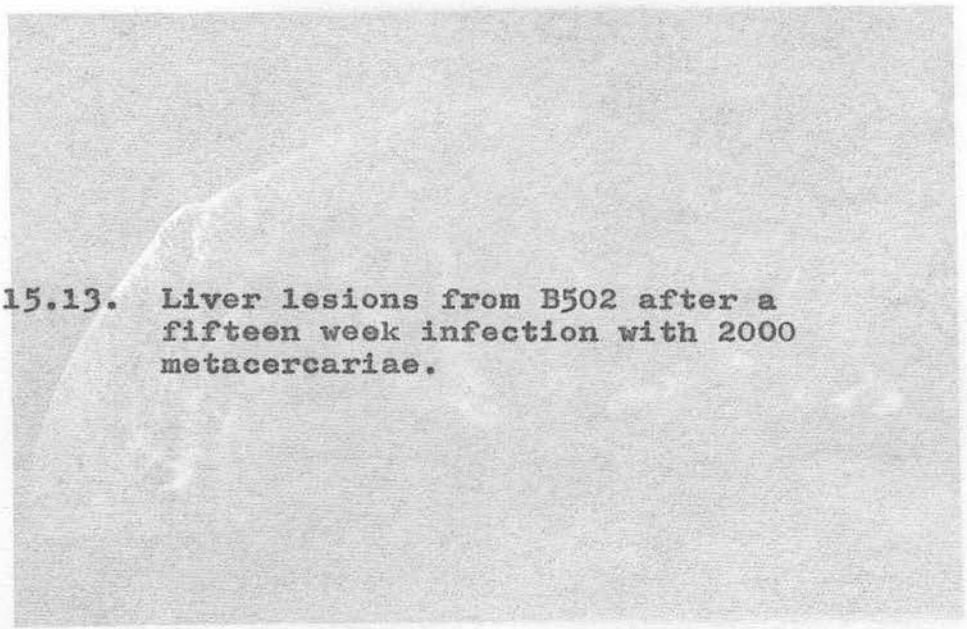


Plate 15.13. Liver lesions from B502 after a fifteen week infection with 2000 metacercariae.

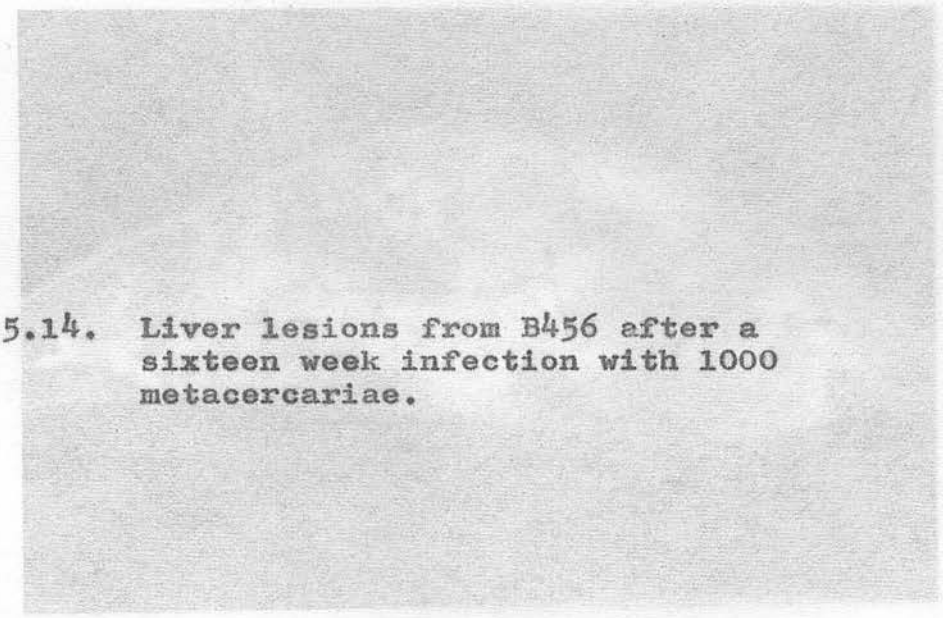
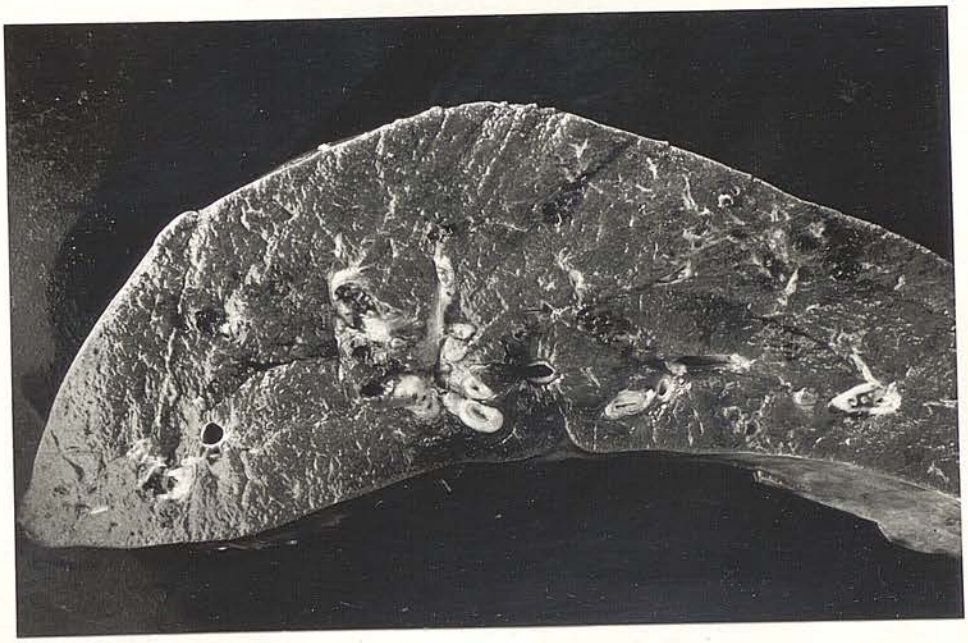


Plate 15.14. Liver lesions from B456 after a sixteen week infection with 1000 metacercariae.



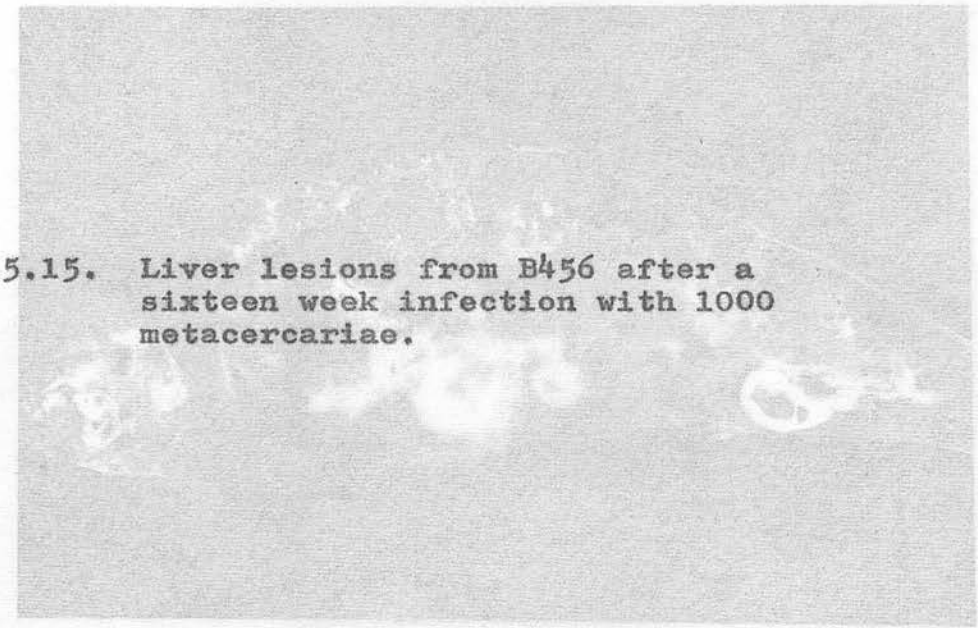


Plate 15.15. Liver lesions from B456 after a sixteen week infection with 1000 metacercariae.

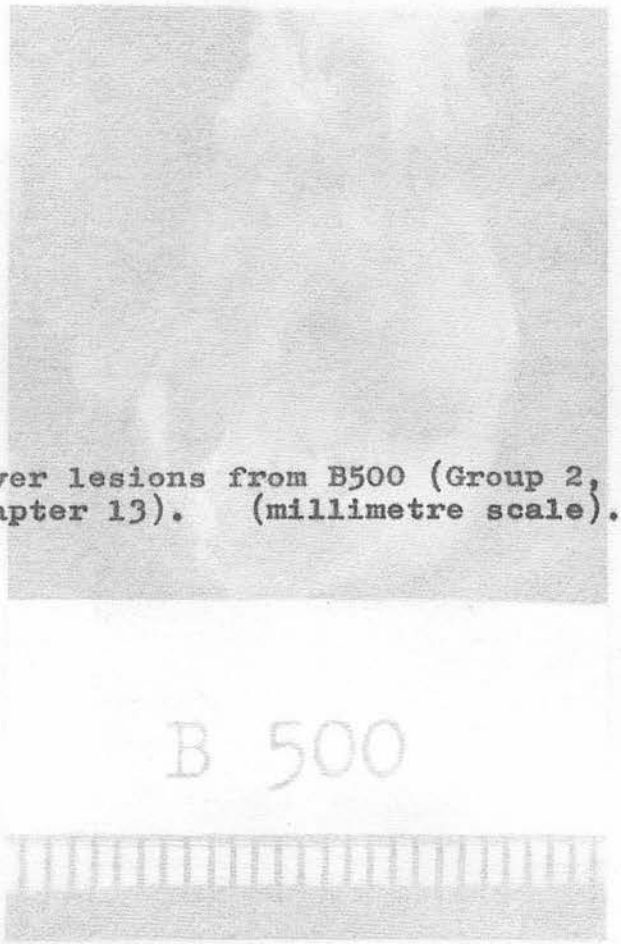
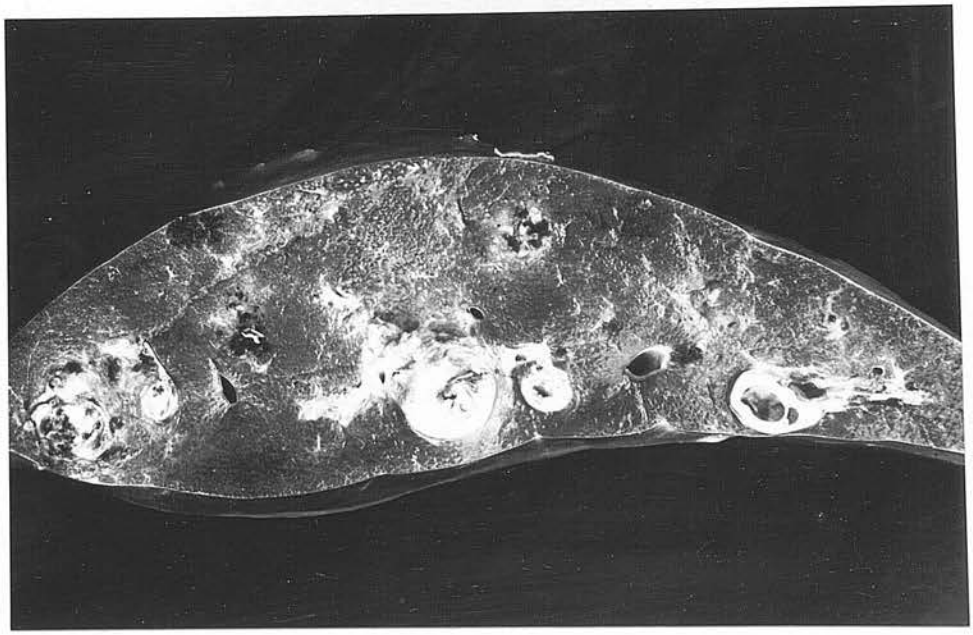
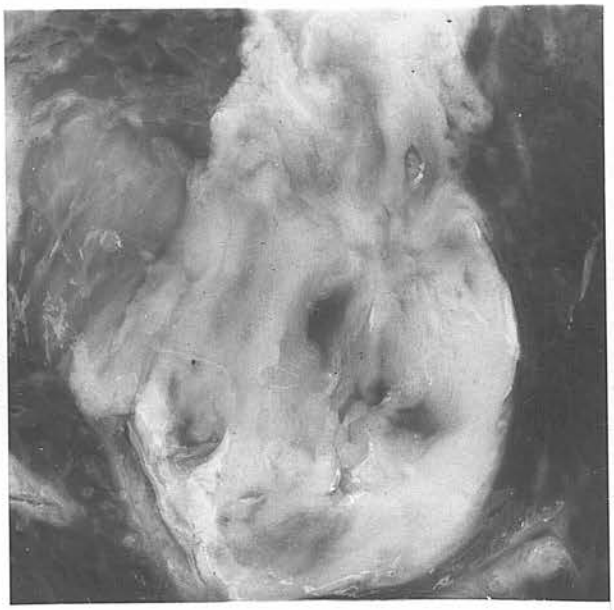


Plate 15.16. Liver lesions from B500 (Group 2, Chapter 13). (millimetre scale).





B 500

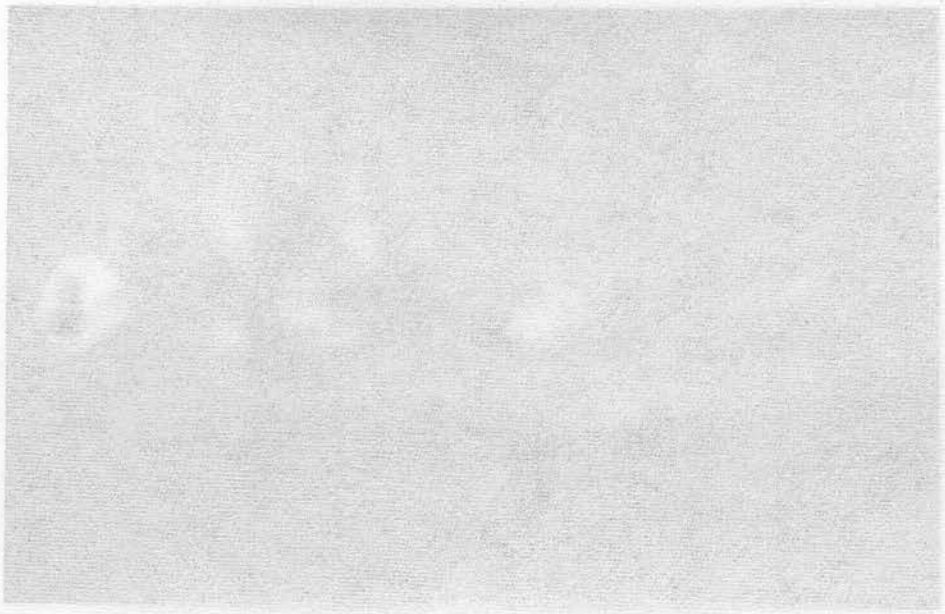
The liver parenchyma was generally more fibrous than in B502, especially the ventral lobe. There were a few holes in the liver capsule where flukes had penetrated. An enlarged and fibrous bile duct was visible on the visceral surface of the ventral lobe, this being the earliest infection in which this was seen.

Eighteen week infections - B506, B509, B512 and B513 (500 metacercariae). The main bile ducts were more enlarged and fibrous and the liver parenchyma was also more fibrous than in the 16 week infection. Some flukes were still in the parenchyma and circumscribed lesions of both types were present. Plate 15.16 shows a fibrous node from another animal with fluke tracks in it. Calcification of the bile ducts was not apparent in any of these 4 livers.

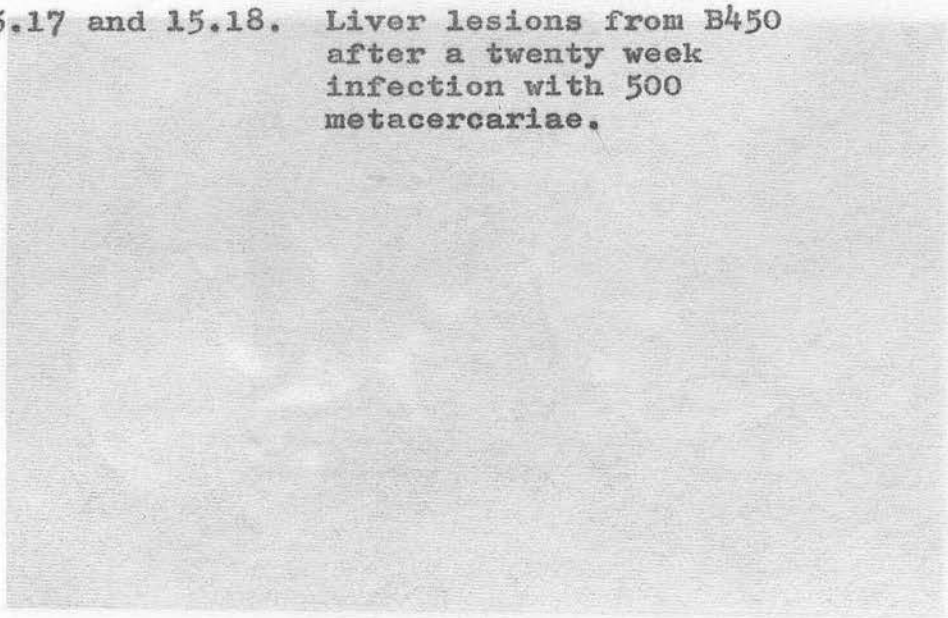
Twenty week infection - B450 (500 metacercariae). There was little difference from the 18 week infections except that even fewer flukes were found in the liver parenchyma, and small areas of calcification were seen in the main bile ducts. Plate 15.17 shows the ventral lobe in section; haemorrhagic lesions are seen together with a very much dilated bile duct. The parenchyma of this part of the liver appeared more fibrotic than the dorsal lobe (Plate 15.18).

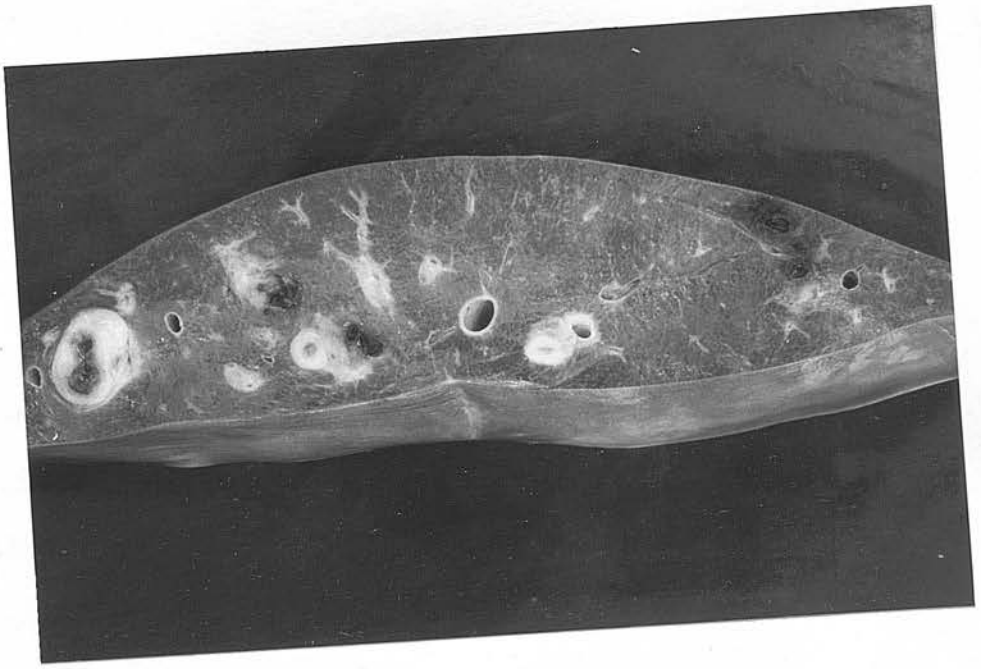
(b) Late chronic infections:

Twenty-four week infection - B455 (500



Plates 15.17 and 15.18. Liver lesions from B450 after a twenty week infection with 500 metacercariae.





metacercariae). This liver appeared to be much more lightly infected than B450, which supports the suggestion that the high proportion of the flukes which were found in the gall bladder had lived there for some time (Chapter 14).

A few flukes were still in the liver parenchyma. Calcification of the main bile ducts was more prominent than in B450 but was still by no means heavy.

Twenty-six week infection - B378 (160 metacercariae). The lesions closely resembled those in B455 except that no recent parenchymatous lesions were found. However some very fibrous fluke nodes were present, including one very large one $2\frac{1}{2}$ cm. in diameter. One of these nodes had calcified areas in it.

Thirty week infection - A805 (700 metacercariae). Fibrosis of the bile ducts and the liver parenchyma was marked especially in the ventral and caudate lobes. There was also extensive calcification of the bile ducts. No recent parenchymatous lesions were seen.

Thirty-seven week infection - B971 (400 metacercariae). The general appearance of this liver was again one of a well developed chronic fascioliasis. A fibrous node immediately under the liver capsule contained a liver fluke, which had recently penetrated the capsule. A few other nodes were found in the liver and some of these also contained live flukes.

The ventral lobe was again more fibrous than the rest of the liver.

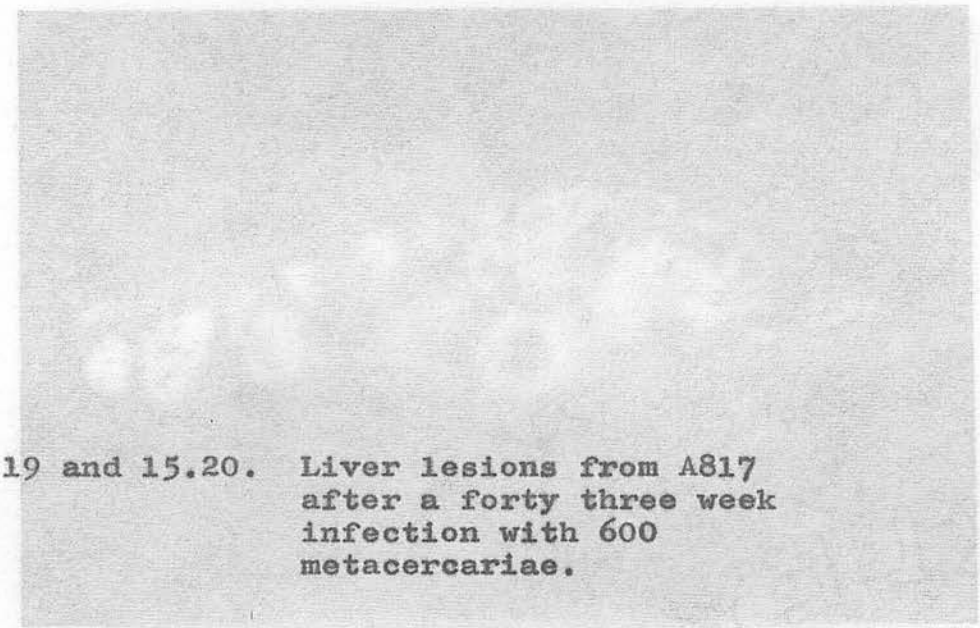
Forty-three week infection - A817 (600 metacercariae). The lesions were confined to the ventral lobe and a band approximately 10 cm. wide along the edge of the liver to the caudate lobe. The rest of the liver appeared normal. No recent parenchymatous fluke lesions were found.

The bile ducts in the affected parts of the liver were very thickened, and there was very marked calcification with complete cylinders lining some bile ducts. Plate 15.19 shows these lesions, together with a fibrous node. Plate 15.20 shows the heavily fibrotic lesions from the visceral surface of the ventral lobe, which was much reduced in size due to contraction of this fibrous tissue.

Fifty-one week infection - B973 (400 metacercariae). The lesions were very similar to A817 except that there was not so much calcification of the bile ducts. The lesions were largely confined to the ventral lobe.

A few nodes containing live flukes were present. These nodes were not heavily fibrotic and such a node, from which a fluke measuring 35½ mm. long was recovered, is shown in Plate 15.21. Other nodes were more fibrous and in these the flukes were found to be dead and degenerate.

Plate 15.22 shows a typical thickened bile duct,



Plates 15.19 and 15.20. Liver lesions from A817 after a forty three week infection with 600 metacercariae.

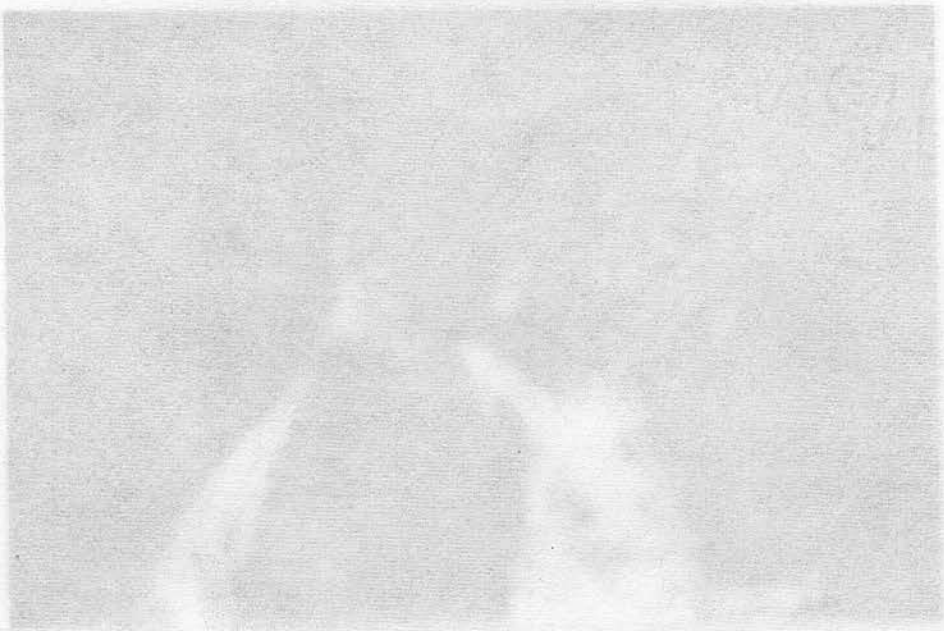
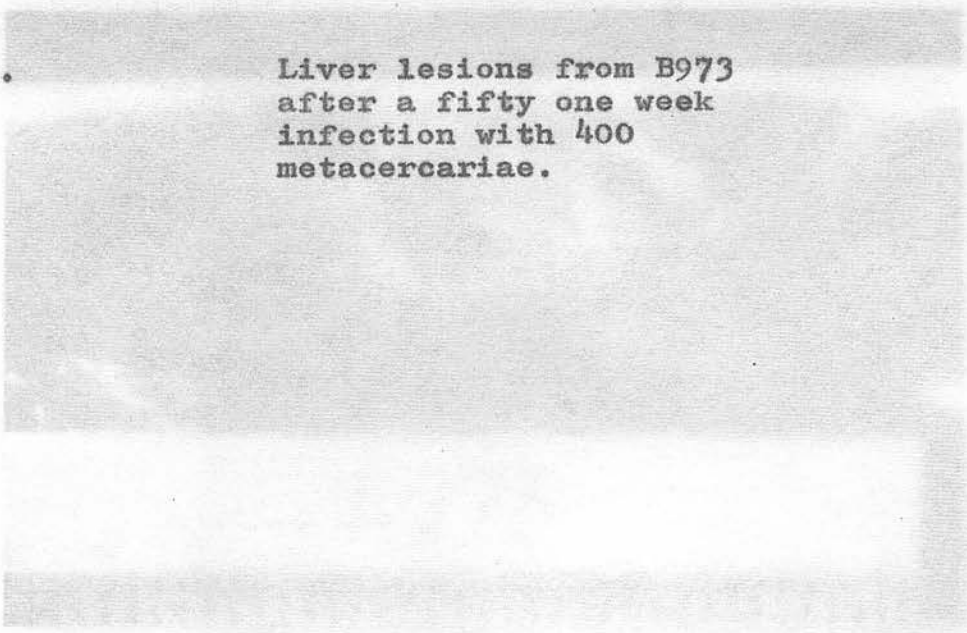
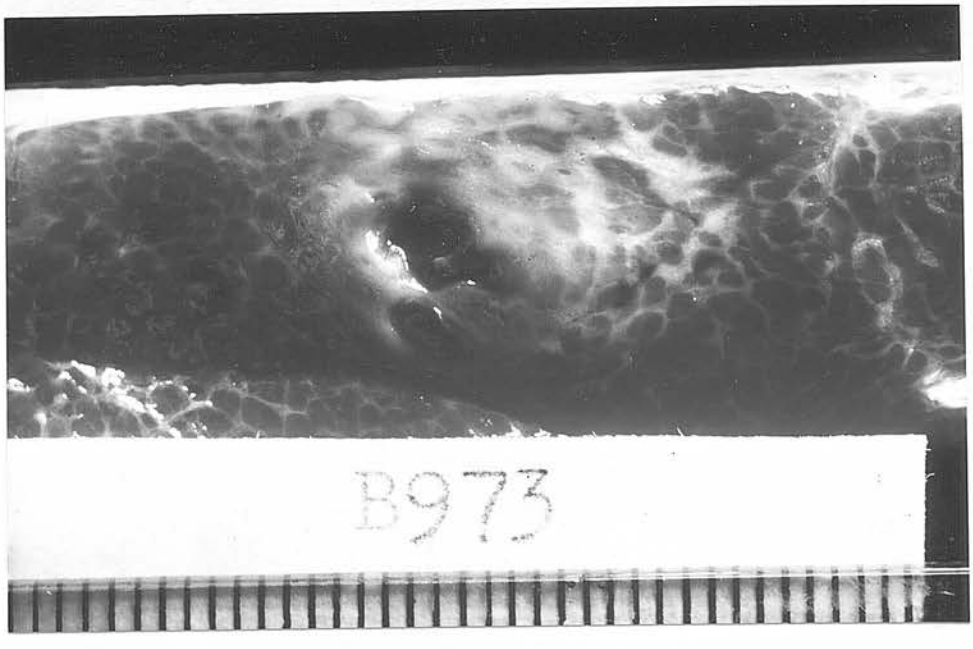
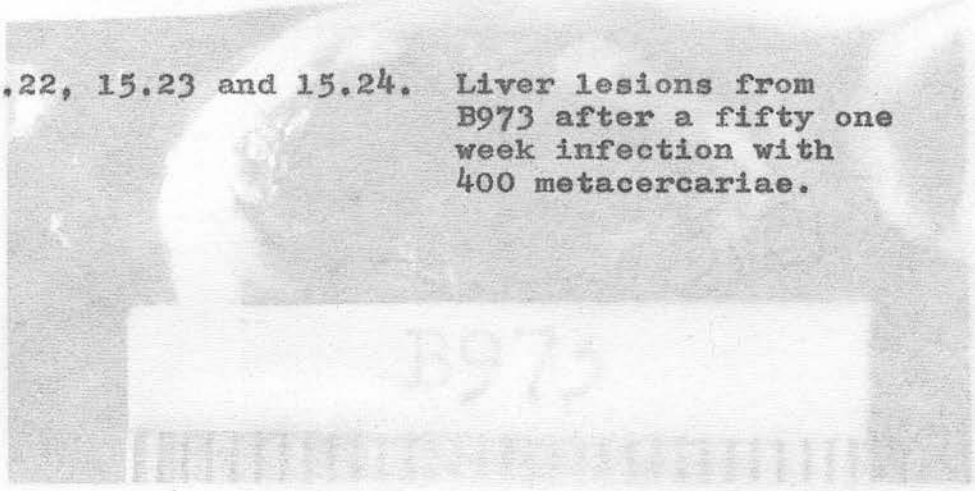


Plate 15.21. Liver lesions from B973 after a fifty one week infection with 400 metacercariae.

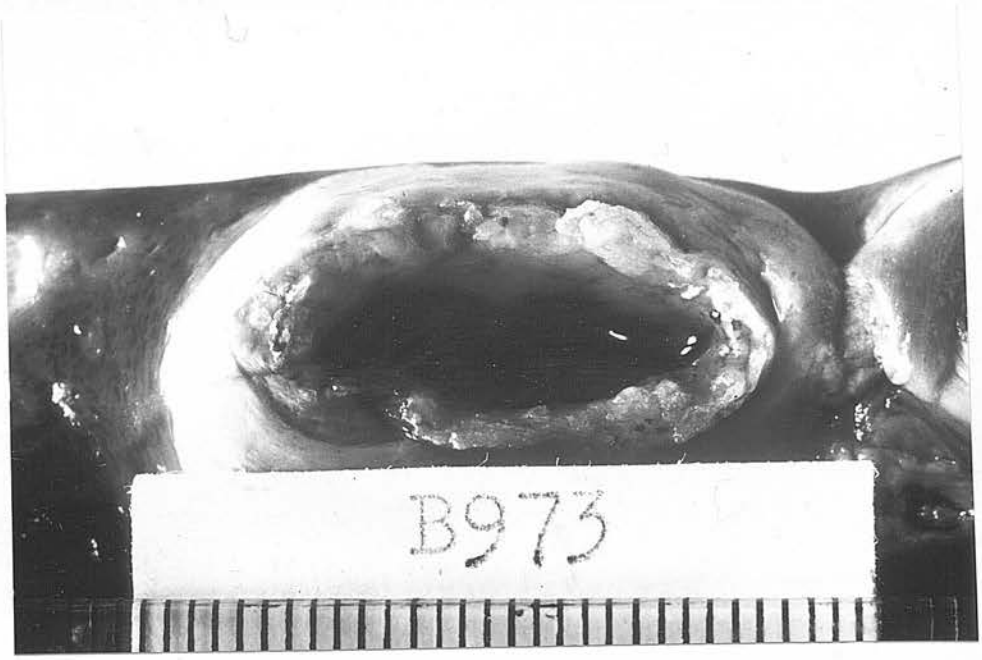



B973



Plates 15.22, 15.23 and 15.24. Liver lesions from B973 after a fifty one week infection with 400 metacercariae.


B973


B973


B973

and Plate 15.23 shows a dilated bile duct, such local dilations being commonly found in F. gigantica infections in cattle. Plate 15.24 is of a section from the more normal part of this liver which shows little evidence of the infection.

3. Challenge infections

(a) Long interval: 8329 was initially infected with 500 metacercariae (Dinnik, personal communication, 1967) 64 months before receiving a challenge infection of 160 metacercariae. It was slaughtered 26 weeks after the challenge infection. No eggs of F. gigantica were found in the faeces from 50 months after the initial infection until the challenge infection became patent.

There were no apparent differences between the lesions found in this animal and those in B378 which also had an infection of the same length with 160 metacercariae. In particular very few nodes were present and all were of the fibrotic type. There was no apparent calcification of the bile ducts.

(b) Short interval: B498, B500, B501 and B504 were each infected with 500 metacercariae and challenged with a further 500 after 10 weeks. They were all slaughtered 18 weeks after the initial infection.

Small areas of calcification were found in the bile ducts of three of the livers, whereas none were found 18 weeks after single infections. Otherwise all

pathological changes caused by the initial infections were similar to those following single infections of the same age. The ventral lobes were again more fibrous than any other areas of the liver.

The lesions from the challenge infections did not differ from single infections of the same age but were largely restricted to the less fibrous parts of the liver.

Discussion and conclusions

The macroscopical appearance of the liver in the 10 week old infection showed the liver damage to be more diffuse than at any other time. However, in most cases the results of the biochemical tests indicated that the most pathogenic phase of the parenchymatous stage of the infection occurred somewhat later than 10 weeks after infection.

There are two possible reasons for this lack of correlation. The flukes start to enter the bile ducts at about 10 weeks after infection but, as they are now relatively large, the sum of the tissue damage may continue to increase for a further 4-5 weeks even though there are fewer flukes in the parenchyma.

It could also be that there is a lag phase between the parenchymal damage and its reflection in the enzyme levels. Stevenson and Wilson (1963) considered that the mechanisms of inactivation and excretion of the liberated enzymes could well be a function of the activity of the liver, so that the increased serum-

enzyme activity could arise from both an increase in enzymic release into the plasma and also an inhibition of the normal rate of excretion from the plasma.

Except for the 2 and 3 week old infections, and those in the late chronic stage, there was little superficial indication that any of the livers were infected, few lesions being found until the livers were sectioned.

Despite the apparently even distribution of flukes in the liver during the parenchymatous stage there is more fibrosis in the ventral lobe, and this becomes more marked as the infection gets older. It is possible that the flukes initially enter the ventral lobe and that the resulting reaction is most severe in that area. However it was found that in C37 the flukes had reached the centre of the liver by two weeks after infection.

It would seem that the fibrous nodes are formed following localized burrowing by individual flukes. This may occur because these flukes had not entered the bile ducts at the usual time and later the larger ducts had become too fibrosed, while the flukes were now too large to enter the small ducts. It is also possible that large flukes are unable to migrate in the liver parenchyma for mechanical reasons, and are therefore restricted to a localized area. There was a general tendency for flukes to become more restricted in their migration range in infections of 6 weeks and over.

The lesions in the calves infected with 1000 metacercariae appear to mature earlier than in those which had been infected with 2000 metacercariae; the prepatent periods were also shorter. These differences may be related to a "crowding" effect similar to that described by Ross, Todd and Dow (1966) and Ross (1967) in high level infections of F. hepatica in calves.

CHAPTER 16

Discussion of Experimental Infections

Introduction

This discussion of the experimental infections, and comparison with the work of other authors, will be largely confined to a consideration of *Fasciola gigantica* infections in cattle. Only limited appropriate reference will be made to *F. gigantica* infections in sheep, or to *F. hepatica* infections in cattle and sheep.

Clinical observations

The clinical effects of the infections of *F. gigantica* in calves differed somewhat from those reported by Bitakaramire (1968, 1969) and Bitakaramire and Bwangamoi (1969) who also worked in Kenya. Their 8-month old calves, infected with either 500 or 1000 metacercariae, appeared to thrive until about 6 weeks after infection, but thereafter their coats became rougher and they appeared relatively dull although they continued to eat. In an experiment lasting 27 weeks the calves which harboured 75-285 adult flukes survived although they were in poor condition and anaemic, but the single calf which had 430 flukes died 23 weeks after infection. The presence of more than 1000 flukes caused death about 12 weeks after infection. From about 18 weeks post-infection the

visible mucous membranes of almost all the calves appeared pale, but diarrhoea, submandibular oedema or pyrexia were not observed. Similarly neither diarrhoea nor submandibular oedema were reported by Guralp, Ozcan and Simms (1964), or by Sewell (1962) except for one animal which had a severe terminal diarrhoea.

In the present work few animals were studied for more than 18 weeks after infection, but clinical symptoms were not seen in any calf which was infected with 1000 metacercariae or fewer.

No comparisons of live-weight gains are possible as the animals' live-weights were not recorded by Bitakaramire (1968) and Bitakaramire and Bwangamoi (1969). Furthermore, as neither the breed nor the management of their calves was described, any direct comparison with their clinical findings is not justifiable. However, their calves were reared parasite-free until 8 months of age and thus it is likely that they were maintained indoors throughout the experiment. This difference in management and feeding may account for the more marked clinical symptoms in their infections, despite lower numbers of flukes than were found in the present work.

It is unlikely that many flukes had died and been lost in animals infected for less than 30 weeks. Thus A805, which had been infected with 700 metacercariae, showed a 55% recovery rate at 30 weeks

after infection and this is much higher than any recorded by Bitakaramire and Bwangamoi (1969) at 27 weeks post-infection.

The effects of temperature and other environmental factors on the pathogenicity of metacercariae have been described by Davtyan (1956) and Boray (1963). However, Bitakaramire (1968) kept his infected snails at a constant temperature of 26°C, little different from that employed in the present studies. It is however possible that storage at 4°C for two months (Bitakaramire and Bwangamoi, 1969) had reduced the infectivity of their metacercariae, but it seems unlikely that this procedure would increase the pathogenicity.

Sewell (1962) infected 10 month old White Fulani (zebu) cattle in Nigeria and concluded that levels of infection of the order of 200 flukes per animal could result in an immediate reduction in growth-rate in animals which have become infected during the time in which they are actively growing. However, it is probable that these animals had earlier carried a much greater fluke burden than that recovered at slaughter 40-42 weeks after infection. Furthermore, in 2 year old bullocks, levels of infection in excess of 300 flukes per animal resulted in a marked loss of condition commencing some 3 months after infection, and ending in death at about 6 months after infection. It is therefore clear that the clinical effect of

F. gigantica was much more marked than seen in the present work or that reported by Bitakaramire (1968) and Bitakaramire and Bwangamoi (1969). It is a matter of conjecture whether this is due to the West African strain of F. gigantica being more pathogenic than that in East Africa, or whether the White Fulani is more susceptible to the effects of the parasite.

Sewell (1962) did not describe the production and storage methods used with the metacercariae used to infect his animals but it is possible that environmental factors may have enhanced their pathogenicity.

A reduced rate of live-weight gain commenced much earlier in Sewell's animals than in those in the present studies, but Sewell observed no other clinical symptoms until the infections became patent. The principal manifestations seen then were a severe anaemia and a continuing loss of weight, while bilirubinaemia was seen terminally (Sewell, 1962).

Chronic F. gigantica infection in lambs was studied by Condy (1961) in Rhodesia. A direct comparison with the sheep infected in these studies is not possible, as Condy used much smaller sheep and moreover only one animal was infected at each dose level. However, Condy concluded that F. gigantica may cause a reduction in weight gain even at the low level of infection used, 9-36 flukes per sheep.

The East African strain of F. gigantica would also

appear to be less pathogenic than that used by Davtyan (1953) in Armenia, U.S.S.R., who reported the death of sheep of comparable size to those used in the present work, infected with as few as 31 and 34 flukes, at 130 and 84 days after infection respectively.

The Russian author, Grigoryn (1958) referred to disturbances in the cardiovascular and central nervous systems of sheep infected with F. gigantica. Such symptoms were not seen in the present work.

Parasitological observations

1. Prepatent periods

The mean prepatent period of F. gigantica for all the single infections in calves was 91±5 days. This period tended to be longer in calves infected with 500 metacercariae or less, 92±4 days, than in those which were infected with 1000 or 2000 metacercariae, 85±4 days.

These results agree closely with those of Bitakaramire (1969), who reported that fluke eggs first appeared in the faeces 12-14 weeks after infection, and Sewell (1962) who gave a mean of 93 days for his calves. Alicata (1938) found the prepatent period in a calf to be 84 days in Hawaii, while Guralp, Ozcan and Simms (1964) reported this to be 89 days in a steer in Turkey and in Armenia, U.S.S.R., Davtyan (1953) noted that F. gigantica needed 101-103 days to reach sexual maturity in cattle.

2. Faecal Fasciola egg counts

The counts of F. gigantica eggs in the faeces were never high in any animal, even in the first few months after patency, and they fell to very low levels after 40 weeks post-infection. A comparison of faecal egg counts between animals is difficult both because of the differences in live-weights, and therefore in the amount of faeces passed daily, and because of the different numbers of flukes which were present. It was however surprising that A805, from which 387 flukes were recovered, had a terminal egg count of less than 10 e.p.g.

The results of these experiments suggest that faecal counts of F. gigantica eggs in cattle are low after about 7-8 months post-infection, over a wide range of infection levels. However, because of the large mass of faeces passed daily, even these low counts may be of importance in the epidemiology of F. gigantica infections. Taylor (1964) estimated that the average adult bovine animal passes about 22 kilogrammes of faeces a day, so that a count of only 5 e.p.g. from such an animal would mean that 110,000 eggs are passed daily. The very low egg counts which were associated with longstanding infections may be caused by nutritional deficiencies in the flukes because of the increasing fibrosis and calcification of the bile ducts.

Taylor (1964) pointed out that liver fluke eggs

accumulate in the gall bladder and are expelled unevenly, thus causing variations in egg counts in the faeces. It is clear from Table 14.2 that there is no correlation between the numbers of eggs in the gall bladder and the concentration in the faeces, even when allowances are made for the difference in the faecal mass from the various animals. Coyle (1961) also found that naturally infected cattle in Uganda had low egg counts, and further that there was no correlation between the egg counts and the fluke burden.

The egg counts reported by Sewell (1966) were much higher than were seen in the present work, the maximum found by him being 505 e.p.g. at 27 weeks after infection. Moreover he considered a count in excess of 100 e.p.g. in cattle to be high. While part of this difference may have been due to a different technique, this cannot explain the results which were obtained by Bitakaramire (1969), who reported an exponential rise in the faecal egg counts, which doubled every 10 days over the first 15 weeks of patent infections and reached levels of around 2000 e.p.g., as the technique he used differed only slightly from that used in the present studies (Bitakaramire, 1967) in which the counts were consistently much lower.

In sheep infected with F. gigantica the mean prepatent period was found to be 95±4 days, rather shorter than the 94-107 days reported by Davtyan (1953) or the 100-105 days found by Grigoryan (1958).

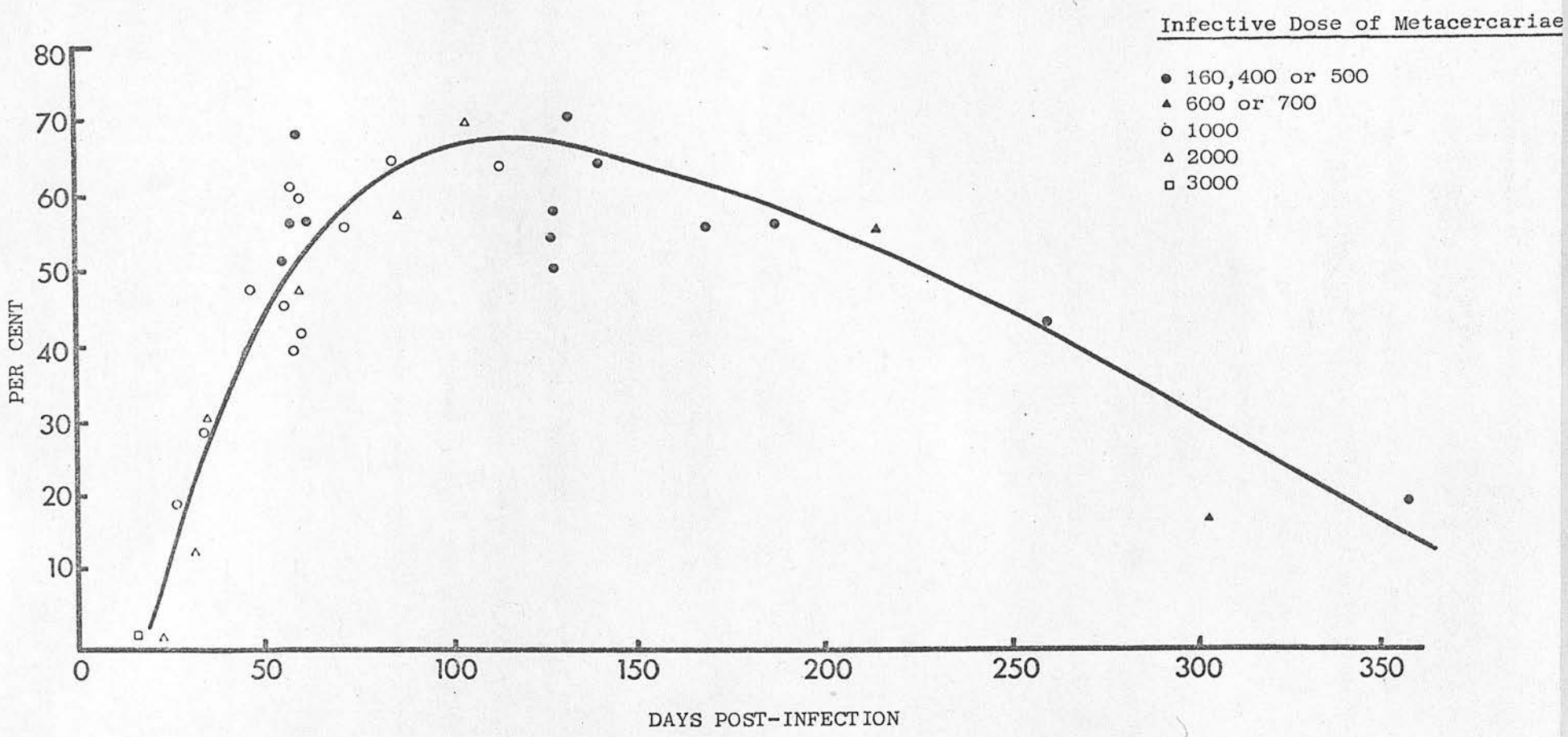
3. Recovery of flukes

The percentage recovery rates from all the experimental infections in cattle are shown in Graph 16.1. The very low recovery rates in the early infections are almost certainly due to technical difficulties caused by the small size of the flukes in the large calf livers. In single infections the recovery rate in all cattle between 10-30 weeks after infection was always more than 55%. This is much higher than the mean figure of 31% from the results reported by Bitakaramire (1969) in infections with 500 and 1000 metacercariae where the experiment was terminated 27 weeks after infection. The highest recovery rate reported by Bitakaramire (of 47%) was well below the lowest rate found in the present work. It is again possible that storage of the metacercariae for a period of two months at 4°C may have been responsible for these relatively poor recovery rates. In another experiment Bitakaramire (1969) failed to infect 22.2% of 27 calves after a single dose of 1000 metacercariae. No such failures occurred at E.A.V.R.O.

Sewell (1966) only infected two animals with a single dose of metacercariae within the range used in the present work and his recovery rates were 35% and 48% (approximately mid-way between the above results) when these calves were slaughtered about 6 months after infection. The very low recovery rates of 10% and 4%

GRAPH 16.1

Recovery of F.Gigantica in Cattle (Single Infections)



in two calves infected with 2600 and 5000 metacercariae respectively, when slaughtered at 40-42 weeks after infection, may well have arisen because some of the flukes died and were expelled before this time especially in view of the earlier high faecal Fasciola egg counts. Such a restricted lifespan for F. gigantica in cattle has been suggested from the experiment reported in Chapter 14.

The mean recovery rate for F. gigantica from sheep which had been given 60 metacercariae each was 62±13%, 7 months after infection. This is much higher than the range in recovery rate of 20-61% reported by Grigoryan (1958), from sheep given 160-270 metacercariae in an experiment lasting 200 days, or by Davtyan (1956) who found a range in recovery of 13-51%.

4. Longevity

These limited studies support the view of Alicata and Swanson (1941) that most F. gigantica are eliminated before the end of one year after infection. Their report that some flukes may survive for at least 40 months is also supported by the observation that the faeces of 8329 did not become negative for F. gigantica eggs until about 48 months after infection, although the egg counts had been very low for some time prior to this (Dinnik, 1967, personal communication).

The percentage recovery of F. gigantica beyond 40 weeks post-infection was far lower than at any earlier time after the infection had become mature (Graph 16.1).

Furthermore, dead and degenerating flukes were found in the three animals with the longest infections, and the heavy liver weights of A817 and B973 may indicate that their infections had been much heavier.

The occurrence of dead and degenerating flukes in 3 of the 10 sheep, only 29 weeks after infection with F. gigantica, may suggest that this fluke does not live so long in sheep as does F. hepatica.

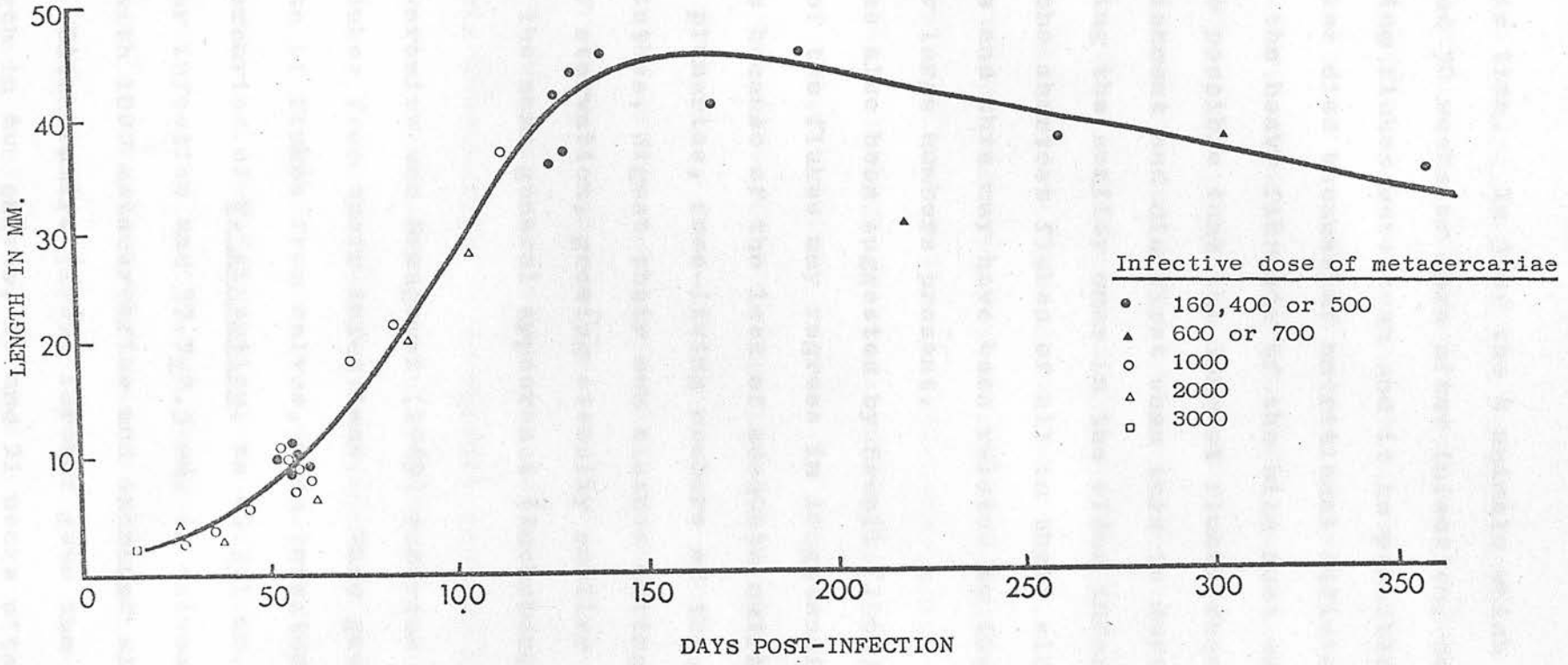
5. Lengths of flukes

The lengths of the flukes from all the single experimental infections in cattle are shown in Graph 16.2. The growth curve follows a sigmoid pattern, being approximately linear from 4 to 20 weeks after infection. The range of mean lengths of mature flukes was 41-47 mm. in single infections in animals killed before 30 weeks after infection. This maximum size was reached 17-20 weeks after infection. The growth rate of the flukes does not appear to vary greatly over the range of infections studied. The mean weekly increase in fluke length between 4 and 20 weeks after infection was 2.7 mm., whereas that given for the period 4-21 weeks after infection by Bitakaramire (1969) was approximately 2 mm. Bitakaramire also found an arithmetic increase (which he incorrectly termed exponential) in the mean fluke length over this period.

There is a tendency for the mean lengths of the flukes recovered more than 29 weeks post-infection to be shorter than those recovered from mature infections

GRAPH 16.2

Growth of F.Gigantica in Cattle (Single Infections)



before this time. In 3 of the 4 animals which were slaughtered 30 weeks or more after infection, dead and degenerating flukes were seen and it is possible that these flukes died because of nutritional deficiencies caused by the heavy fibrosis of the bile duct wall. It is also possible that the largest flukes require the most nourishment and die first when this is deficient, thus leaving the smaller ones in the older infections. A805 had the shortest flukes of all in these older infections and this may have been related to the relatively large numbers present.

It has also been suggested by Sewell (1962) that the size of the flukes may regress in longstanding infections because of the lack of adequate nutrition. Moreover, planarias, free-living members of the Phylum Platyhelminthes, digest their own tissues during periods of starvation, growing steadily smaller although retaining the same general appearance (Buchsbaum, 1951).

Bitakaramire and Bwangamoi (1969) reported rather smaller flukes from their infections. They gave the mean length of flukes from calves, each infected with 500 metacercariae of F. gigantica, as 43.3±1 mm. at 27 weeks after infection and 37.7±2.5 mm. in calves infected with 1000 metacercariae and examined slightly earlier. Bitakaramire (1969) further gave the mean fluke length in two calves, 20 and 21 weeks after infection, as 33.0 mm. and 34.2 mm. respectively.

Post-mortem examinations and gross pathology

The pathological findings described by Sewell (1962) in mature single infections closely resembled the lesions which were found in the livers in the present study. However, in the two animals which he slaughtered in extremis, anaemia was a marked feature but this was not seen in any of the calves at E.A.V.R.O., though B502 showed signs of a mild anaemia. One of Sewell's other two calves had a similar anaemia but the second showed no such condition. Furthermore, two of his animals also had marked oedema of the intestinal wall, which was not seen in any of the calves at E.A.V.R.O.

The main differences in the liver lesions reported by Sewell from those seen in the present studies were the absence of calcification in the bile ducts of two of his four animals and the presence of marked adhesions between the liver and the adjacent organs in three of his four cattle. He further refers, in two of his animals, to some purple-blue swellings under the liver capsule, which released a dark brown fluid when cut open. It is likely that these were locally dilated, relatively thin-walled, bile ducts such as have been referred to in Chapter 15.

Natural infections in Uganda were studied by Coyle (1961) who found that the macroscopic lesions were mainly confined to the ventral lobe in long-standing infections and, in the majority of cases,

these consisted of nothing more than one or two white bands, about 2-4 cm. broad, overlying the main bile duct and its larger branches. In severely affected livers the liver tissue was often distinctly pale and hyperplasia was seen as a thickening of the central and dorsal parts of the organ. These are very similar findings to those in experimental infections at E.A.V.R.O., where hyperplasia of the liver was seen in all mature infections, being earlier in the more heavily infected animals. In severe infections Coyle found numerous flukes in the common bile duct including the intestinal branch, suggesting that they may be reaching the end of their life, or may have lost their hold through mechanical difficulties such as the accumulation of phosphatic casts, or may have been crowded into this abnormal site; or a combination of these factors. He also found that in old infections, encrustations were frequently found and sometimes formed complete casts of the ducts, similar to those found in A817. Fibrosis was heaviest in the vicinity of the bile ducts, but in severe cases it was noticeable throughout the liver tissue. The difference between the dorsal and ventral lobes as regards the visible damage and the number of flukes present was very marked, in many cases even where the ventral lobe had greatly enlarged bile ducts, the dorsal part of the liver was unaffected or only slightly affected. This was also seen in long-

standing infections at E.A.V.R.O. Coyle (1961) suggests that the reason for this distribution of lesions in the liver is that the bile ducts in the dorsal lobe are small and branch frequently, so that fewer flukes can find suitable sites there. He found that it was always the dorsal lobe which was enlarged through hyperplasia, whilst the ventral lobe tends to be atrophied in heavy infections.

Bitakaramire and Bwangamoi (1969) also found in their experimental infections that the fibrosis chiefly affected the ventral lobe of the liver. Bitakaramire (1969) studied the development of the lesions in 8 month old calves, from 1-31 weeks after infection with 1000 metacercariae of F. gigantica. He reported finding solitary and confluent, irregularly-shaped, mottled plaques (2-5 mm. in diameter) in the subcapsular regions of the liver at 3 weeks post-infection and similar elongated dark red plaques 3-5 mm. in diameter on the surface of the liver in 4 week old infections, neither of which were seen in the E.A.V.R.O. calves. Otherwise his description of the gross pathology in the early infections closely resembles that seen in the present work.

In 7 week old infections, Bitakaramire found diffuse, pale and congested areas on the surface of the liver, and at 9 weeks after infection he found grossly diffuse, red lesions of up to 5 mm. in diameter extending from the capsule into the parenchyma up to

about 10 mm. deep. No direct comparison is possible at 10 weeks after infection, but it is likely that the diffuse red lesions seen by Bitakaramire at 9 weeks after infection are the early stage of the diffuse liver damage which was seen at the later time at E.A.V.R.O.

In the early chronic phase of the disease, at 15 weeks post-infection, Bitakaramire found that the liver surface was irregularly raised by dark-brown lesions which contained flukes. These areas were possibly locally dilated bile ducts such as were seen in the infected calves at E.A.V.R.O. The liver capsule had also ruptured in many areas at this time, but this was never seen in the present study. By 16 weeks after infection, Bitakaramire found cirrhosis in the ventral lobe but little in the rest of the liver. This was somewhat later than that seen macroscopically at E.A.V.R.O.

The picture given by Bitakaramire (1969) from then on closely resembles that found in the present study, except that calcification of the bile ducts was not seen until 31 weeks after infection, whereas it was found at 15 weeks after infection in B502 at E.A.V.R.O. However, it cannot be ruled out that microscopic calcification had occurred earlier than 31 weeks after infection, as the livers were not examined histologically at 20, 21 or 26 weeks. Another surprising difference is that Bitakaramire did not

observe fresh fluke tracks later than 16 weeks post-infection, whereas these were prominent in all infections up to 24 weeks after infection at E.A.V.R.O.

The only observations in the present studies on the gross pathology of F. gigantica infections in sheep were on those infected 7 months before with 60 metacercariae (Chapter 11). In Turkey, Guralp, Ozcan and Simms (1964) found that the walls of some of the fluke-containing bile ducts in sheep were enormously thickened, being up to more than one centimetre in width, but that calcification was not seen. In the sheep at E.A.V.R.O. the bile ducts were enlarged but the walls were only up to 2 mm. thick, and again, calcification was not seen.

Similar findings were reported by Sogoyan (1955, 1958) in the U.S.S.R.

Resistance and the response to challenge infections

Natural resistance to infection with Fasciola, and the work which has been carried out in an effort to produce an adequate protection against infection with Fasciola spp. have been reviewed briefly in Chapter 3. There are very few reports of attempts to produce a resistance to F. gigantica in cattle, and in none of these has a single low level infection of normal metacercariae been used. For this reason the results of workers who used repeated low level infections or naturally acquired infections will be considered.

In a survey on slaughter cattle in the Lake Region of Tanzania in 1961 most of the livers infected with F. gigantica had a low fluke burden, 81.2% containing only 0-50 flukes. These were all long-standing infections with very fibrotic livers. Similar figures were reported by Bitakaramire (1968) in a larger survey in Kenya, who considered that because of the severity of cirrhosis observed in the livers, it seemed likely that the original fluke burden was considerably higher. Further, he considered that since almost all Kenya beef cattle spend their entire lives on one farm, it would appear that age immunity, or more likely, acquired immunity to re-infection had occurred.

These two observations, suggesting that some resistance may have developed against the continuous re-infection to which many of these cattle were undoubtedly subjected, are supported by Coyle (1961). He found at Entebbe Abattoir, Uganda, many livers which showed typical lesions of longstanding fascioliasis but which contained no flukes. These cattle were in the 5-8 year old age group and all originated from the Teso district, where they were subjected to re-infection with F. gigantica throughout their lives. Coyle suggested that such cases represent evidence of the establishment of some barrier to re-infection. However, he does not make it clear whether he was referring to a purely physical barrier or to the

establishment of a protective immune reaction. Sewell (1966), in his reference to this work of Coyle, thought it perhaps more likely that the purely physical barrier afforded by the gross fibrosis is responsible for the absence of re-infection.

Sewell (1966) tested the possibility in Nigeria that resistance may develop following prolonged exposure to repeated low levels of infection. He found some evidence for this in a steer infected with 20-25 metacercariae of F. gigantica on 5 days each week for 16 weeks. When this animal was alaughtered, two distinct populations of flukes were found in the liver; no forms of intermediate size were observed, and the numbers of the two types present were not consistent with a steady rate of infection and development. Although a slowly developing immunological resistance could cause such an imbalanced population, Sewell points out that this could also have been due to other causes such as a change in the liver parenchyma which might render it a less suitable environment for development, or the onset of fibrosis in the walls of the bile duct preventing the later flukes from entering the lumen of the ducts at the correct time. Alternatively the metacercariae used towards the end of the experiment may have been of lower infectivity than those used earlier, but Sewell considered that this was unlikely.

A similar approach to that used by Sewell (1966)

was also tried by Bitakaramire (1969) who exposed his calves to natural infection, in an area where fascioliasis was endemic, for six months. When the calves were passing 16-520 Fasciola e.p.g. faeces they were housed, treated with hexachlorophene, and thereafter maintained under fluke-free conditions. After a further period of 8 weeks the calves were again dosed with hexachlorophene, and 4 were slaughtered and examined to test the efficacy of treatment. The remaining calves were each challenged with 5000 metacercariae of F. gigantica, and they were slaughtered 11 weeks after the challenge infection when the flukes were recovered and counted. The percentage recovery resulting from the challenge infection of 5000 metacercariae of F. gigantica ranged from 0.66 to 5.6. No control calves were infected, so the results are subject to the proviso that the challenge metacercariae were of normal infectivity. Bitakaramire assumed that this was so as the metacercariae were produced under similar conditions to those used to infect calves in earlier experiments, where the recovery had ranged from 13% to 47% after single infections using from 500 to 10,000 metacercariae. Bitakaramire concluded that after cattle infected with F. gigantica have been treated with anthelmintics they may exhibit a high degree of resistance to challenge infection.

Similar results were obtained by Boray (1967) who

infected 4 zebu x Jersey steers with 1000 metacercariae each of F. hepatica and, after treatment with an appropriate anthelmintic, challenged each animal, together with the same number of previously uninfected controls, with 5000 metacercariae of F. hepatica. Boray reached the same conclusions as Bitakaramire (1969), but he also considered that a high degree of acquired resistance developed in cattle after the infection was eliminated or reduced by spontaneous recovery. Boray further concluded that a considerable tissue reaction and intensive fibrosis may be necessary to produce such resistance to re-infection. These conditions may be achieved by an initial heavy infection resulting in extensive fibrosis of the liver parenchyma during the early migration phase, or by moderate infections resulting in chronic cholangitis. The preferential migration of young flukes into the ventral and left lobes was considered by Boray (1967) to facilitate the formation of a host barrier by concentrating the fibrosis, which probably results in immobilization and destruction of the migrating flukes.

No such preferential migration by young flukes was seen in F. gigantica infections in the present work, although the ventral lobe became progressively more fibrotic than the rest of the liver after the flukes had entered the bile ducts. The bile ducts which were apparently favoured by the flukes were in this lobe and in the central part of the liver.

The results of the studies on challenge infections at E.A.V.R.O. have already been discussed in Chapter 13. The slight - but statistically significant - reduction in the recovery of flukes from the challenge infection, as compared with the single infection in the control animals, may have been due to an immunological resistance but it could also have arisen merely because the young flukes were harder to recover from the much more fibrous parenchyma of the livers in those animals which had been infected twice.

It would, however, seem unlikely that cellular reaction plays much part in resistance to re-infection, because these animals were re-infected at about the time of maximum tissue reaction from the primary infection, in order to test this hypothesis, It is also likely that the initial infection of 500 metacercariae was not sufficient, in the time available, to produce the high degree of tissue reaction and intensive fibrosis which Boray (1967) considered may be necessary to produce resistance to infection. Certainly the fibrosis was not particularly obvious macroscopically and furthermore it was observed that the flukes from the challenge infection preferentially migrated in the less fibrous parts of the liver. A heavier infection, and longer period before the first infection was terminated, was used by Boray (1967) and it is therefore possible that the livers of his calves were more fibrotic when they were re-infected.

Ross (1967) believed that the essential basis for differences in natural resistance to F. hepatica between different species is the difference in fibrous structure of the liver, and Ross, Dow and Todd (1967) believed that the naturally fibrous nature of the liver parenchyma in the pig is a major factor contributing to this host's resistance to F. hepatica infections.

It may be possible to test the hypothesis that resistance to Fasciola is mainly or solely due to fibrosis of the liver parenchyma, by the use of chemicals or plants, which are known to cause hepatic cirrhosis, before infection with metacercariae. It might also be possible to induce sufficient fibrosis in this way to give a large measure of protection against natural infections.

Only a single animal was available to study the effects of F. gigantica infections at long intervals. This animal, 8329, was challenged with 160 metacercariae 64 months after an initial infection of 500 metacercariae and over a year after that infection had ceased to be patent. The challenge infection was given at the same time, and from the same batch of metacercariae, as the single infection in B378 (Chapter 14). Although the prepatent periods of the infections in these two animals were similar, the F. gigantica faecal egg counts were always much lower in 8329 than in B378 (Table 14.2). This may be partly explained by the fact that 8329 was a much

larger animal than any other in these experiments, so that the daily bulk of faeces was greater. However, the number of F. gigantica eggs recovered from the gall bladder was also much less than in any animal except those with infections of over 40 weeks duration, and the percentage recovery was also far below all other infections of comparable duration, again only exceeding those in the two animals with the longest infections (Graph 16.1). On the other hand the mean length of the flukes recovered from 8329 was of the same order as that from B378 and the other animals which had infections of similar duration.

This low percentage recovery of flukes from 8329 was unlikely to be due to a low infectivity of the metacercariae, as the same techniques were used in all the other cattle and B379 which was infected from the same batch of metacercariae had a much higher fluke burden. However 8329 was a very much older animal than any other used in these experiments so there may have been some true age-resistance. Also its liver may have been more fibrous, following the ingestion of plants containing pyrrolizidine alkaloids, and other factors, and so less suitable for F. gigantica to migrate in. The possibility of a true age-resistance is supported by Boray (1967) who showed that steers aged 14-17 months were more resistant to initial heavy infections than younger animals. Finally, as 8329 had previously been infected, it is likely that there was

residual fibrosis from this primary infection.

It was found by Keck and Supperer (1967) that as soon as a section of bile duct in cattle becomes free from F. hepatica, the calcifications begin to break off and are carried away, healing usually being complete within a year. Calcification was not found in 8329 at 26 weeks after the challenge infection, whereas it was found in the other cattle which had late chronic infections. It is assumed that calcification had been present from the primary infection, but that it had not yet formed from the challenge infection because of the smaller fluke burden. However other factors may have been involved.

Treatment of young cattle, during their first infection with F. hepatica, with an anthelmintic efficient against both mature and immature flukes would, according to Boray (1967), prevent serious clinical symptoms of the disease. The treated animals would develop strong resistance to subsequent infections without severe liver damage, but, if re-infection does not take place within approximately one year the cattle again become susceptible. However further studies will be necessary before these findings can be applied to F. gigantica in cattle.

Haematology

In the experimental infections of cattle with F. gigantica at E.A.V.R.O. only one animal, B502, which had a fluke burden of 1387 when it was slaughtered at

15 weeks post-infection, developed anaemia. This anaemia was not severe and moreover it was not possible to assess the part played by the fluke infection due to the presence of a heavy co-existing burden of Haemonchus spp. and because an Eperythrozoon infection was seen during the last few days of the experiment. Nevertheless the onset of the anaemia had been progressive before the Eperythrozoon spp. appeared in the peripheral blood. Although anaemia was not present in any of the animals in the long-term experiment at the time of slaughter, it is possible that it had developed earlier in the longest infections but that recovery had taken place as flukes died and were shed.

These results are surprising in view of those obtained by Bitakaramire (1969) where the mean P.C.V.s of his two groups of calves, with mean fluke burdens of 171±14 and 287±59 when slaughtered 27 weeks post-infection, fell gradually from 14 weeks after infection to reach terminal levels of 22% and 18% respectively. The anaemia was normocytic and normochromic but was not accompanied by a reticulocytosis. Anaemias similar in type but much more severe were reported by Sewell (1966), where fluke burdens of 325 and 492 in two animals led to P.C.V.s of 6% and 12% at 25-28 weeks after infection. Another animal, which was infected with 2600 metacercariae, developed a similar degree of anaemia followed by a slow recovery, but a fourth

animal, infected with 5000 metacercariae, showed only very mild symptoms. The number of flukes recovered from these latter two animals at 40-42 weeks after infection was 259 and 197 respectively, but, as has already been discussed, it was likely that their burdens had been much higher earlier in the infection.

There are several possible reasons for the marked difference between these results. The possibility that the strains of F. gigantica used by Sewell (1962) and Bitakaramire (1969) were more pathogenic than that at E.A.V.R.O. has already been referred to. Perhaps the more severe anaemia which was seen in their animals was another expression of this. The possible effect of temperature and other environmental factors on the pathogenicity of metacercariae has also already been referred to. Breed differences in the hosts are another possible reason for the dissimilarity in the degree of anaemia seen.

Boray (1967) observed that calves in particularly good condition showed more resistance to F. hepatica infections than poorer animals. It is therefore possible that the differences in management and feeding may have affected the pathogenesis of the F. gigantica infections also. Sewell (1962) maintained his calves indoors, and it is likely that Bitakaramire (1969) also kept his calves indoors, whereas the calves at E.A.V.R.O. had daily access to good pasture. This appears to be the only major difference in the

management of the animals and it may be that the good grazing at E.A.V.R.O. enhanced the resistance of the calves to the pathogenic effects of F. gigantica.

The only report on the leucocytic changes in F. gigantica infections in cattle is by Bitakaramire (1969). A rise in the mean total leucocyte count was seen in calves during the first month after single infections of 500 or 1000 metacercariae, and this agrees with the results at E.A.V.R.O. Thereafter there was some fluctuation but a further slight rise was apparent at about 13-16 weeks after infection with the 500 metacercariae dose level, which was more marked at the 1000 metacercariae dose level, and this was followed by a gradual fall. A similar temporary increase in the total leucocyte count was seen at E.A.V.R.O. from 12-15 weeks post-infection in calves with single infections of 1000 or 2000 metacercariae, but thereafter the fall was steep. However, no definite trends were seen in the calves infected with single doses of 500 metacercariae.

Bitakaramire (1969) demonstrated an eosinophilia in calves with single infections of 500 and 1000 metacercariae of F. gigantica, but the levels were not as high as those in similar infections at E.A.V.R.O., and the mean counts in his animals started to rise somewhat later. Maximum levels were however reached at a similar time, 13 weeks post-infection. There was also a marked tendency for the eosinophil counts to

fall after these maximum levels had been reached, at all levels of infection, both at E.A.V.R.O. and in the calves infected by Bitakaramire.

The peak in the total mean lymphocyte count at 6 weeks and the low count at 13 weeks after infection found by Bitakaramire (1969), in calves with single infections of 500 or 1000 metacercariae of F. gigantica, were not seen at E.A.V.R.O. Moreover the low total mean neutrophil counts, seen by Bitakaramire at both 6 and 16 weeks after infection at both dose levels, were not observed at E.A.V.R.O.

Although no other reports are available on the leucocyte patterns in F. gigantica infections in cattle, there are several references to infections with this parasite in sheep. Thus Grigoryan (1953) found no definite correlation between acute fascioliasis and the total leucocyte count. Grigoryan (1958) also found that eosinophilia was first seen some 8-10 days after infection in both acute and chronic infections. This eosinophilia remained constant at 35-40% of total leucocytes, and was accompanied by a moderate leucocytosis and a monocytosis of 3-4% of the total leucocyte count.

The leucocyte picture in F. hepatica infections in cattle was studied by Ross, Todd and Dow (1966) who found a significant rise in the eosinophil counts 3 weeks after infection, which persisted throughout the experimental period of 23 weeks. Changes in the

numbers of the other leucocytes were less marked, but a peak occurred in the total count at 16 weeks after infection, which was attributed to a rise in lymphocytes and neutrophils.

Furmaga and Gundlach (1967) studied the leucocyte response in F. hepatica infections in sheep and found that the total leucocyte count was much higher than normal during the acute stage of the disease, but only slightly higher in the chronic phase. Slight neutropenia and pronounced lymphopenia occurred, particularly early in the disease. High eosinophilia was observed during the whole experiment, but no clear changes were noticed in the basophils and monocytes.

The variations in the leucocyte counts in F. hepatica infections in sheep were also studied by Sinclair (1962) who found that a progressive leucocytosis began soon after the administration of the metacercariae. A peak occurred at 9 weeks after infection which was followed by a fall to about preinfection levels at 14 weeks post-infection. The total leucocyte count then fluctuated at about this level until the experiment was terminated. An absolute eosinophilia was mainly responsible for the leucocytosis, but there was also a slight increase in the number of lymphocytes. However both the lymphocyte and the neutrophil counts began to fall from about 13 weeks after infection, until 24 weeks after infection, after which the neutrophil counts increased

as the lymphopenia progressed.

It is clear from the results in the experiments in Edinburgh and at E.A.V.R.O., and from this comparison with the results of other workers, that the only clearly defined change occurring in the leucocyte picture in fascioliasis of sheep and cattle is a consistently high eosinophilia. Considerable variations occur in the numbers of lymphocytes and neutrophils present, but monocytes and basophils only fluctuate within a normal range.

Serum biochemistry

The biochemical procedures had to be carried out on sera which had been stored at -25°C. All enzyme activities decay to some extent during prolonged storage, even at this low temperature, so the results should be interpreted with this in mind.

Reichard and Reichard (1958) found that ornithine carbamyl transferase (O.C.T.) could be stored for more than a year at -15°C without any demonstrable decrease in enzyme activity, and furthermore that repeated freezing and thawing and slight haemolysis did not influence O.C.T. activity. In none of the serum samples assayed however was there any visual evidence of haemolysis. Sewell (personal communication, 1967) considered that little deterioration of glutamate oxaloacetic transaminase (G.O.T.) activity would occur in sera stored at -25°C for the periods necessary in this work.

Either Ford and Lawrence (1965) or Roberts (1968) or Ford, Ritchie and Thorpe (1968) or Patterson and Sweasey (1966) had previously carried out all the biochemical procedures which were used in this work at E.A.V.R.O., except for alkaline phosphatase assays, on sera stored at -20°C for unstated periods. Furthermore, Patterson (personal communication, 1970) stated that glycoprotein analyses would still be valid after storage at -25°C for 18 months, and Amador and Wacker (1963) found that alkaline phosphatase is stable at -20°C for 20 months.

The biochemical procedures used at E.A.V.R.O. were chosen with this necessity for storage in view. These included 3 enzymes known to have a high concentration in the liver (O.C.T., G.O.T. and sorbitol dehydrogenase (S.D.)), which might be expected to be present in increased concentrations in serum from animals in which liver damage had occurred. Total bilirubin and alkaline phosphatase were selected in order to assess liver function, while the iodine flocculation test was chosen because it had previously been shown to be a satisfactory indicator of non-specific liver damage in acute F. hepatica infection in sheep (Chapter 9). Moreover this test can be rapidly carried out in the field, with a minimum of equipment and reagents. Alterations in the level of total serum protein and protein fractions have been shown to occur in fascioliasis by several workers but had been little

studied in F. gigantica infections.

The results from each of these biochemical procedures will be considered separately.

(1) G.O.T.

There are no previous reports of serum G.O.T. levels in F. gigantica infections. Considerable increases were seen in the infected calves at all three levels of infection. The very few exceptions to these uniform results have already been referred to.

G.O.T. may be derived from a wide range of body tissues (Cornelius, Bishop, Switzer and Rhode, 1959; Boyd, 1962) as well as from the liver, and increased serum G.O.T. levels are commonly associated with damage to muscle cells, but there is no evidence of material damage to any organ other than the liver in fascioliasis. Thorpe and Ford (1969) thought it likely that leakage of G.O.T. is associated with damage to the stromal tissue and with the damage caused to the bile ducts by adult flukes. In experimental F. hepatica infections in sheep they found that whereas the serum G.O.T. level was still rising in some of the infected groups when observations ceased at 11½ weeks after infection, there were no significant changes in serum G.O.T. activity in some of the other groups of infected sheep. Thorpe and Ford further considered that the flukes themselves may be another source of the enzyme, released either at death or during their normal metabolic activity.

Only slightly increased G.O.T. activities were observed by Ross, Todd and Dow (1966) in two calves each infected with 1300 metacercariae of F. hepatica, but significant increases were reported in F. hepatica infected cattle by Nickolic, Nickolic, Nevenic, Bugarski, Pavlovic, Ciric, Mladenovic and Polic (1962). Conversely, no such increases were found by Hankiewicz and Hankiewicz (1964) or Valcarenghi and Molinari (1959) on similarly infected cattle. Further, Rossow, Rittenbach, Urbaneck, Wick and Krause (1966) did not consider G.O.T. levels of value in the diagnosis of medium or light F. hepatica infections in cattle.

(2) O.C.T.

There are no previous reports of serum O.C.T. levels in F. gigantica or F. hepatica infections. Much larger increases in the serum concentrations than were seen with G.O.T. occurred in the calves at all three levels of infection. The very few exceptions to these uniform results have been referred to earlier.

Ford (1965) reported a relatively high activity of this enzyme in liver. Of five other tissues examined only the small intestine contained significant levels. He found an increase in the concentration of this enzyme in the serum of cattle with dimidium bromide poisoning and in sheep poisoned by repeated doses of carbon tetrachloride and by sporidesmin, while Ford, Ritchie and Thorpe (1968) found similar results in

calves poisoned with ragwort (Senecio jacobea).

(3) S.D.

Most of the animals infected with F. gigantica showed large increases in serum S.D. concentration, but in one animal which had been infected with 500 metacercariae there were very slight increases over preinfection levels.

There are no reports of S.D. levels in F. gigantica infections, but Thorpe and Ford (1969) found significant increases in the serum levels in sheep given single doses of 2000 metacercariae or 3 successive doses of 200 metacercariae of F. hepatica. As the peak of activity occurred within the period of intrahepatic fluke migration, they considered that the enzyme leakage is probably a direct effect of parenchymal cell damage. Ford (1967) found that in cattle the liver had the greatest activity, followed by kidney.

(4) Alkaline phosphatase

While there were no marked differences between the infected and the control calves in the experiment described in Chapter 12, there were increases in the infected animals in the experiments described in Chapter 13. In view of these conflicting results the study would need to be repeated before any valid conclusions could be drawn.

According to Stevenson and Wilson (1963) alkaline phosphatase is present in many tissues, particularly in

the liver, kidney, intestinal mucosa and growing bone. It is said to be excreted into the bile by the liver and the plasma activity can therefore by used as an indication of the excretory function of the liver, as any obstruction to the biliary system may give rise to increased plasma activity.

There is one report of alkaline phosphatase levels in experimental fascioliasis in which Pinkiewicz and Madej (1967) reported increases in the serum concentration of this enzyme in sheep. Ford and Boyd (1962) found that serum alkaline phosphatase levels gave no reliable indication either of hepatocellular damage or of excretory dysfunction in two cows after parenteral administration of dimidium bromide.

Garner (1952) found that although there is a significant increase in serum alkaline phosphatase concentration in severe generalized liver disease, the wide range observed in normal cattle precludes the use of assays of this enzyme as a reliable diagnostic aid in liver disease. Garner's studies were concerned with only single observations on individual animals. However it was found at E.A.V.R.O. that, although there were wide ranges in normal levels between individual animals, the levels in each control animal were uniform over the period of the experiment.

(5) Protein-bound hexose

There were considerable increases in serum protein-bound hexose at all three levels of infection.

No reports are available on serum protein-bound hexose levels in fascioliasis,

The non-specificity of increases in serum glycoprotein concentrations is well known, increases being found in Johne's disease, chronic mastitis and actinomycosis by Patterson and Sweasey (1966). Winzler (1955) also referred to a wide range of conditions in man in which significant increases have been found in protein-bound hexose.

(6) Bilirubin

Only very slight changes in serum bilirubin concentration were observed with the lower two levels of infection. However the one animal which was infected with 2000 metacercariae and which survived more than 12 weeks after infection showed a marked increase from this time until the experiment was terminated at 15 weeks post-infection.

Sewell (1966) found a large increase in serum bilirubin concentrations in the later stages of sub-acute F. gigantica infection in a steer. He also found smaller, but still marked, terminal increases in two steers with fluke burdens of 325 and 492 respectively. Bitakaramire and Bwangamoi (1969) reported that the carcases of three calves with F. gigantica burdens of 2000-3000 were icteric, but did not state the serum bilirubin levels. Marked bilirubinaemia was reported by Grigoryan (1958) in about 50% of sheep with acute or sub-acute F. gigantica

infections but was less frequent with the chronic infection.

In general the reports of bilirubinaemia in cattle with fascioliasis are rather conflicting. Thus Rossow, Rittenbach et al. (1966), and Ross, Todd and Dow (1966) reported that serum bilirubin estimations were not of value in the diagnosis of light or medium infections. Furthermore, Sinclair (1967) failed to demonstrate raised bilirubin levels in sheep infected with F. hepatica but did not state the fluke burdens. It would therefore seem that serum bilirubin is not a sensitive or a reliable indicator of liver dysfunction in ruminants except perhaps in heavy infections.

(7) Iodine flocculation

Fairly consistent positive results were obtained at the three levels of infection in all animals by 9-14 weeks after infection. Occasional very early positive results would seem to indicate that a score of more than 4 is necessary to establish a positive result especially if the results may have been affected by prolonged storage. One animal, however, had a consistent score of 12 from a week after infection.

No reports are available of this test being used in F. gigantica infections in cattle, but similar results were reported by Ross, Todd and Dow (1966) in two calves with F. hepatica infections, while Hankiewicz (1965) found that about 80% of cattle

infected with F. hepatica gave a positive result. Ross (1967) also found positive results in sheep with natural infections of F. hepatica, and considered that this test indicates a reduced serum albumin concentration. However Paton (1969) thought that the flocculation tests probably depend on a disturbance of the balance between the various protein fractions of the serum, rather than on quantitative or qualitative abnormalities in various globulins as was originally thought. Paton further stated that as well as liver diseases, other diseases in which hyperglobulinaemia is a feature also give positive results.

(8) Total and fractional serum protein concentrations

The total serum protein concentration increased at all three levels of infection, and highest concentrations were seen at 13-16 weeks after infection. There were no significant differences in the albumin concentrations at the 500 metacercariae level of infection but the concentration fell in the calves which were infected at the 1000 and 2000 metacercariae levels. Much of the increase in total serum protein in the infected calves was due to an increase in the gamma globulin concentration, although there were also some increases in the alpha and beta fractions.

Serum protein estimations were carried out by Sewell (1966) on two animals infected with 300-500

F. gigantica. In one of these the total serum protein levels remained within normal limits but the albumin-globulin ratio fell considerably, whereas in the other the total serum protein concentration fell below normal by about 17 weeks after infection, but the albumin-globulin ratio remained fairly constant. Weinbren and Coyle (1960) reported on the variations in the serum protein levels in naturally infected and control cattle. They observed a highly significant fall in the albumin-globulin ratio with an increase in the gamma globulin concentration, but no significant change in the total serum protein concentration.

The changes in the serum proteins in F. hepatica infections have been studied by many workers, and are similar in cattle and sheep. The initial hyper-globulinaemia, which is due mainly to an increased gamma globulin concentration, is accompanied later by hypoalbuminaemia which tends to persist during the presence of the adult flukes in the bile ducts. These workers include Ehrlich, Forenbacher, Rijavec and Kurelac (1960); Ross, Todd and Dow (1966) and Hankiewicz (1965) for cattle, and Sinclair (1962), Ibrovic and Gall-Palla (1959), Roberts (1968) and Furmaga and Gundlach (1967) for sheep.

Reid, Armour, Jennings and Urquhart (1970) state that the initial elevations in serum gamma globulin levels in fascioliasis are probably the result of two factors: (a) an immunological response to the invasion

of metacercariae, and (b) a generalised increase in reticulo-endothelial activity associated with liver damage (Paton, 1969). The subsequent fall in the serum protein concentration has been shown by Holmes, Dargie, Maclean and Mulligan (1968) to be associated with hypercatabolism of albumin and globulin due to loss of protein into the bile ducts of the infected animals. The liver is the sole source of albumin (Paton, 1969).

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Appendix Table 9.A Haematological results, erythrocyte series

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	Sheep No.	Preinfection		Weeks Post-infection														
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Packed cell volume (%)	11	34	34	33	31½	31	27	22	20½	20	19½	20½	17½					
	12	38	35	37	39	38½	33	27½	23½	19	18							
	13	42½	39	38	39	37	34½	31	30½	25	23	19	17	13				
	14	42	39	38	40½	38	35	30½	33	31	29½	28	24	22½	21½	18	15½	5½
	15	42	39	39	35	35½	36	34½	37½	36½	37	41	43	43½	39	41	40½	36
Red Cells (million/cmm.)	11	11.80	10.90	11.00	11.00	10.70	8.75	6.75	6.10	6.65	7.55	6.12	5.40					
	12	11.10	9.80	10.20	11.10	11.00	9.75	7.60	7.00	5.90	5.40							
	13	12.30	11.30	10.80	11.60	10.80	9.60	8.50	8.25	7.60	7.00	5.40	4.80	3.50				
	14	12.00	10.40	11.00	11.40	9.60	9.65	7.75	8.60	7.95	8.22	8.50	7.00	7.10	6.20	5.45	4.66	2.06
	15	11.00	10.20	11.00	10.90	10.20	10.40	8.90	9.40	9.45	9.70	11.40	11.60	13.60	11.05	11.90	12.86	12.29
Haemoglobin (G/100 ml)	11	12.3	11.3	10.6	10.6	10.5	8.9	7.8	6.2	6.5	5.8	6.4	5.0					
	12	12.7	11.4	11.5	12.1	11.4	9.8	9.2	6.8	5.3	5.6							
	13	14.0	12.7	12.8	12.1	12.8	8.9	10.3	9.9	7.7	6.7	5.2	4.7	3.7				
	14	14.0	12.9	13.1	12.3	11.7	10.5	9.9	9.2	9.8	8.4	8.7	7.1	7.1	6.5	5.0	5.1	1.7
	15	14.0	12.5	13.4	11.1	11.7	10.8	11.8	11.2	11.2	11.1	13.1	13.3	13.2	12.1	13.2	13.3	12.5
Mean Corpuscular Volume (c.μ.)	11	28.8	31.2	30.0	28.6	28.9	30.8	32.6	33.6	30.1	25.8	33.5	32.4					
	12	34.2	35.7	36.3	35.1	35.0	35.7	36.7	33.6	32.2	33.3							
	13	34.5	34.5	35.2	33.6	34.2	35.9	36.5	36.9	32.9	32.8	35.2	35.4	37.1				
	14	35.0	37.5	34.5	35.5	39.6	36.3	39.3	38.4	38.9	35.9	32.9	34.3	31.7	34.7	33.0	33.3	26.8
	15	38.2	38.2	35.4	32.1	34.8	34.6	38.8	39.9	38.6	38.1	35.9	37.1	31.9	35.3	34.4	31.5	29.3
Mean Corpuscular Haemoglobin (μμg)	11	10.4	10.4	9.6	9.6	9.8	10.2	11.6	10.2	9.8	7.7	10.4	9.2					
	12	11.4	11.6	11.3	10.9	10.3	10.6	12.3	9.7	8.9	10.4							
	13	11.4	11.2	11.8	10.4	11.8	9.3	12.1	12.0	10.1	9.6	9.6	9.8	10.5				
	14	11.7	12.4	11.9	10.8	12.2	10.9	12.8	10.7	12.3	10.2	10.2	10.1	10.0	10.5	9.2	10.9	8.1
	15	12.7	12.2	12.2	10.2	11.5	10.4	13.3	11.9	11.8	11.4	11.5	11.5	9.7	10.9	11.1	10.3	10.2
Mean Corpuscular Haemoglobin Concentration (%)	11	36.2	33.2	32.1	33.6	33.9	32.9	35.4	30.2	32.5	29.7	31.2	28.6					
	12	33.4	32.6	31.1	31.0	29.6	29.7	33.4	28.9	27.9	31.1							
	13	32.9	32.6	33.7	31.0	34.6	25.8	33.2	32.4	30.8	29.1	28.4	27.6	28.4				
	14	33.3	33.1	34.5	30.4	30.8	30.0	32.4	27.9	31.6	28.5	31.1	29.6	31.5	30.2	27.8	32.9	30.2
	15	33.3	32.0	34.3	31.7	32.9	30.0	34.2	29.9	30.7	30.0	31.9	30.9	30.3	31.0	32.2	32.8	34.7

Appendix Table 9.B Haematological Results, Leucocyte Series

Sheep No.		Preinfection		Weeks Post-Infection 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
11	Neutrophils (band)		0	0		0							0					
	Neutrophils (segmented)		1400	539		392							1269					
	Eosinophils		0	25		808							1108					
	Basophils		0	0		25							0					
	Lymphocytes		4116	4288		3528							2943					
	Monocytes		84	48		147							80					
	Total leucocytes	5200	5600	4900	5700	4900	6500	7800	5900	6000	5400	6100	5400					
12	Total leucocytes	8000	6900	8100	9000	8200	6300	6900	6000	6300	8400							
13	Neutrophils (band)		0			0						0	0					
	Neutrophils (segmented)		2400			1595						1920	3595					
	Eosinophils		40			2420						960	1106					
	Basophils		0			0						0	0					
	Lymphocytes		5480			6985						3488	3121					
	Monocytes		80			0						32	79					
	Total leucocytes	8000	8000	7800	9700	11000	10400	9200	9400	6900	7800	6400	7900	9600				
14	Neutrophils (band)		0	0		40									0	75	0	163
	Neutrophils (segmented)		2106	1376		1200									2793	1416	994	2990
	Eosinophils		78	102		1280									1225	745	134	0
	Basophils		0	0		0									98	0	0	0
	Lymphocytes		5577	3596		5400									5488	4916	4166	3054
	Monocytes		39	26		80									196	298	81	293
	Total leucocytes	8300	7800	5100	6900	8000	8000	7200	5000	10900	9400	6700	8100	8700	9800	7450	5375	6500
15	Neutrophils (band)		0	0		0						0			0	0	0	0
	Neutrophils (segmented)		1674	649		1073						1290			1418	1658	1245	780
	Eosinophils		124	0		82						150			32	195	130	150
	Basophils		0	0		0						30			0	33	33	0
	Lymphocytes		4340	5163		4290						4440			4598	4518	4978	4920
	Monocytes		62	88		55						90			252	96	164	150
	Total leucocytes	9700	6200	5900	5200	5500	5200	5400	7400	6000	5200	6000	6800	7100	6300	6500	6550	6000
11		20	50	60	920	990	1130	2020	1060	1840	1680	1455	1030					
12		0	300	240	2030	1390	1110	990	1200	1000	1460							
13	Eosinophils	0	80	140	1320	2140	1210	1170	1640	1410	980	1125	1050	810				
14		20	60	40	690	1130	1490	1340	1000	1210	990	890	1350	2000	1760	852	100	0
15		10	50	30	100	90	120	80	160	150	100	110	130	170	85	100	105	148

All results expressed in numbers of cells per cubic millimeter of blood.

Appendix 10A Fasciola eggs per gram. faeces

	Sheep No.	Weeks Post-infection																		
		11	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	30	31
Total egg count. Results recorded in Graphs. 10.1 and 10.2	23	1	23	64	136	408	288	456	528	672	560	936	824	816	536	424	704	232	448	
	25	9	8	24	96	64	168	200	144	216	240	264	240	576	160	200	240	184	-	272
	30	0	16	8	8	24	16	32	24	40	32									
	20	19	104	136	152	232	344	320	328	328	352	360	312	416	216	360	288	392	680	
	17	25	195	432	296	632	568	1520	824	840										
	24	6	80	32	192	128	144	264	168	288	296	304	160	752	296	448	328	272	464	
Eggs counted in the standard method	23	1	23	64	136	408	288	256	464	560	424	776	680	440	368	128	472	96	256	
	25	9	8	24	96	64	168	160	104	160	192	200	192	512	112	144	208	128	-	216
	30	0	16	8	8	24	16	32	8	24	32									
	20	19	104	136	152	232	344	136	248	232	296	296	240	328	152	264	256	312	672	
	17	25	195	432	296	632	568	1408	752	768										
	24	6	80	32	192	128	144	248	160	208	224	248	104	632	144	408	320	224	424	
Eggs found on a second cover slip	23							200	64	112	136	160	144	376	168	296	232	136	192	
	25							40	40	56	48	64	48	64	48	56	32	56	-	56
	30							0	16	16	0									
	20							184	80	97	56	64	72	88	64	96	32	80	8	
	17							112	72	72										
	24							16	8	80	72	56	56	120	152	40	8	48	40	
Eggs found floating in the counting chambers	23																40	80	64	
	25																32	88	-	72
	20																48	96	376	
	24																152	208	368	

Appendix Table 10B Haematological Results, Leucocyte Series

Sheep No.		Preinfection		Weeks Post-Infection									
				1	4	7	10	13	16	19	23	27	31
13	Neutrophils (band)	42	0	0	0	53	0	26	64	57	59	64	33
	Neutrophils(segmented)	2562	1678	1888	1674	1590	2552	1173	3296	1768	1800	1824	1022
	Eosinophils	252	244	236	135	106	122	0	128	485	384	352	67
	Basophils	0	60	0	0	53	80	25	0	26	30	0	0
	Lymphocytes	5292	4057	3599	3402	3445	5184	3774	2752	3278	3420	4096	2111
	Monocytes	252	61	177	189	53	162	102	160	86	207	64	117
	Total leucocytes	8400	6100	5900	5400	5300	8100	5100	6400	5700	5900	6400	3350
23	Neutrophils (band)	22	0	0	0	82	0	67	56	47	48	41	100*
	Neutrophils (segmented)	1232	1323	2108	1913	1845	2094	1575	1148	1033	1560	1189	1140
	Eosinophils	66	243	589	1725	1845	2173	1106	700	470	312	369	560
	Basophils	0	27	0	36	0	40	0	0	0	0	0	20
	Lymphocytes	2992	3726	3503	3713	4387	3514	3818	3612	2985	2688	2460	2100
	Monocytes	88	81	0	113	41	79	134	84	165	192	41	80
	Total leucocytes	4400	5400	6200	7500	8200	7900	6700	5600	4700	4800	4100	4000
25	Neutrophils (band)	36	0	0	0	129	0	90	107	82	81	60	58
	Neutrophils(segmented)	3182	2893	2867	1278	2387	1245	1395	4761	1435	1133	990	1463
	Eosinophils	107	534	1136	8307	3870	5764	1395	428	1558	486	390	293
	Basophils	0	89	0	0	0	65	45	0	41	0	0	0
	Lymphocytes	3825	5296	4960	4402	6450	6026	5985	5136	5002	6359	4560	4007
	Monocytes	0	88	137	213	64	0	90	268	82	41	0	29
	Total leucocytes	7150	8900	9100	14200	12900	13100	9000	10700	8200	8100	6000	5850

Sheep No.		Preinfection		Weeks Post-infection								
				2	5	8	11	14	17	20	25	31
14	Neutrophils (band)	0	33	0	38	49	0	40	70	63	66	22
	Neutrophils (segmented)	3120	1365	1421	1938	2474	1462	1500	1435	1292	1947	836
	Eosinophils	104	98	0	76	97	68	0	35	220	66	88
	Basophils	0	0	0	0	0	34	0	0	0	0	0
	Lymphocytes	6864	4906	4205	5434	7033	4998	6320	5425	4599	4422	3366
	Monocytes	312	98	174	114	47	238	40	35	126	99	88
	Total leucocytes	10400	6500	5800	7600	9700	6800	7900	7000	6300	6600	4400
30	Neutrophils (band)	54	33	0	0	0	0	40	86	76		
	Neutrophils (segmented)	1431	878	2268	1488	2394	767	1620	1806	1216		
	Eosinophils	135	163	162	1920	1512	1314	648	129	418		
	Basophils	27	65	0	0	63	37	0	0	38		
	Lymphocytes	3699	5265	5589	6096	8505	5182	5792	6450	5548		
	Monocytes	54	96	81	96	126	0	0	129	304		
	Total leucocytes	5400	6500	8100	9600	12600	7300	8100	8600	7600		
20	Neutrophils (band)	0	58	28	0	37	0	48	38	43	39	0*
	Neutrophils (segmented)	1846	2900	1092	1575	1544	1292	960	703	817	1209	468
	Eosinophils	182	145	1260	1228	1286	1666	408	266	753	330	414
	Basophils	0	0	28	32	74	0	48	38	43	20	18
	Lymphocytes	3094	2668	3108	3402	4409	3808	3192	2698	2558	2243	2538
	Monocytes	78	29	84	63	0	34	144	57	86	59	162
	Total leucocytes	5200	5800	5600	6300	7350	6800	4800	3800	4300	3900	3600

Sheep No.		Preinfection		Weeks Post-infection								
				3	6	9	12	15	18	20	25	30
17	Neutrophils (band)	0	99	0	43	65	74	0	64	30		
	Neutrophils (segmented)	2684	1287	900	1403	3380	1517	2108	1088	1800		
	Eosinophils	0	33	2088	3103	3445	1850	1462	512	390		
	Basophils	0	0	0	85	0	37	0	0	0		
	Lymphocytes	6234	5082	4068	3825	6110	3811	3026	4672	3660		
	Monocytes	182	99	144	43	0	111	204	64	120		
	Total leucocytes	9100	6600	7200	8502	13000	7400	6800	6400	6000		
24	Neutrophils (band)	62	28	26	0	0	45	76	60	56	41	49
	Neutrophils (segmented)	2356	1210	1170	1365	819	968	1368	880	616	841	1600
	Eosinophils	62	192	1378	1855	3159	698	1976	80	924	185	243
	Basophils	0	0	0	35	0	0	0	0	0	0	0
	Lymphocytes	3627	3905	2548	3605	3822	2723	4066	2880	3948	2993	2837
	Monocytes	93	165	78	140	0	66	114	100	56	40	121
	Total leucocytes	6200	5500	5200	7000	7800	4500	7600	4000	5600	4100	4850

	Sheep No.	Pre-infection		Weeks Post-infection																											
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Eosinophils	13	150	50	210	70	70	80	70	140	80	80	110	60	60	90	70	70	0	40	40	160	340	920	890	130	200	220	240	310	180	180	100	-	70
	23	20	140	470	2040	2740	1650	1830	2930	2140	1240	2175	1930	1230	1170	1090	1030	580	690	280	270	380	360	290	100	140	310	230	160	330	510	710	330	
	25	70	260	800	950	2040	6540	5360	4490	3440	3150	4645	4080	2650	3390	1260	2160	550	590	210	740	1560	1670	980	405	280	580	470	350	370	680	450	-	365
	14	50	120	110	60	50	85	85	120	100	80	110	60	30	70	70	40	90	70	70	90	90	170	380	330	220	240	100	120	210	120	80	-	65
	30	30	110	70	190	300	860	2090	890	920	1240	1385	1160	1010	650	340	580	260	200	70	40	450	900	270										
	20	110	70	190	790	780	1470	925	1720	1890	1300	2680	2120	1320	590	500	410	400	250	350	140	170	510	400	235	290	180	240	170	180	80	120	215	
	17	80	52	310	1560	2200	3200	3000	3100	1580	2075	2860	3310	2180	1560	1860	3050	2120	1030	700	210	120	140											
	24	80	80	210	610	1020	2970	3510	1660	1760	2275	2700	3280	1880	1250	810	1360	1810	350	560	80	170	1030	780	370	230	270	140	120	130	140	210	220	

* The last examination took place 30 weeks after infection.

All results expressed in numbers of cells per cubic millimeter of blood.

Appendix Table 10C Haematological Results, Erythrocyte Series

	Sheep No.	Pre-infection	Weeks Post-infection																												
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Packed Cell Volume (%)	13	41	43	39½	35½	35½	36	37	37½	34½	38½	37	36	39	40	36	40	36	36	38	36½	36	35	36	33	35	38	38	41½	35	35	39½	-	38½
	23	37	42½	38	36½	35½	35	37	33½	33	33	31	29	27	28	30	30	27	29	26	23½	24½	23½	21	17½	18	16	17½	17	18	18	22	22	
	25	35½	39½	38	33½	35	37	34½	33	33½	33	35½	33½	34	34	34	34	33	33	35	33	33½	33	35	37½	37	37	36	33½	31	34½	32½	-	35
	14	39½	41	39	36½	37½	36½	36	34½	36	37½	37½	35	39	38	40	39	36	35	35	37	36	38	37	37½	37	36	36½	38	37	37½	39	-	39½
	30	40½	41	34½	34	34½	34½	35	30	31½	34	35½	31	36	34	35	35	33	33	35	32½	35	34	34										
	20	37	39	33½	32½	33½	34½	34	32½	30½	31	28½	26	28½	24	26	27	27	25	26	26	28	27	28	31	27	27½	26	27	25	25½	28	28½	
	17	32	33½	34	33	32	33	31	29	29	29½	29	26½	24	24	23	23	19	18	15½	14½	11½	11											
	24	37	37	35	33	34	32½	34	37	34½	34½	35	32	31	33	32	29	29	27	29	27½	27	31	28	29½	30	31	28½	27½	26	25	28	31½	

On weeks 16 and 17 post-infection the Improved Neubauer Counting Chamber was used for the erythrocyte count.

*The last count was at 30 weeks post-infection in the case of sheep 23 and 20, and at 21 weeks for sheep 30. That shown under week 20 for No.24 was done at week 21 post-infection.

Red Cells (million/c.m.m.)

Sheep No.	Pre-infection	Weeks Post-infection												
		1	4	7	10	13	16	19	23	27	31			
13	12.60 12.49	12.24	9.82	10.54	10.88	10.88	10.17	10.60	10.92	10.80	11.02			
23	10.84 14.54	10.84	9.55	9.04	8.54	8.70	7.65	6.76	5.16	5.22	6.07*			
25	10.98 12.16	11.28	9.92	9.42	9.50	10.10	9.13	11.30	10.30	8.78	9.56			

Sheep No.	Pre-infection	Weeks Post-infection									
		2	5	8	11	14	17	20	25	31	
14	12.11 13.54	10.88	10.72	11.42	11.70	11.96	10.20	11.88	10.98	11.84	
30	12.34 11.32	10.44	10.02	10.64	11.48	11.28	10.80	10.27*			
20	11.78 11.84	9.68	9.96	9.12	8.44	8.12	6.80	6.76	7.58	7.58*	

Sheep No.	Pre-infection	Weeks Post-infection								
		3	6	9	12	15	18	20	25	30
17	9.82 11.24	9.54	8.84	8.81	7.86	5.54	4.36	3.28		
24	11.78 10.14	9.76	10.50	9.22	9.38	8.90	7.86	8.06*	7.96	10.46

*The record shown under week 20 for sheep 30 and 24 was taken at week 21 post-infection.

Haemoglobin (g/100ml)

Sheep No.	Pre-infection	Weeks Post-infection												
		1	4	7	10	13	16	19	23	27	31			
13	13.3 13.7	13.1	11.8	10.9	11.4	11.5	11.3	12.0	12.3	12.1	12.4			
23	13.4 13.5	12.0	10.7	9.7	8.6	9.5	8.4	6.6	5.2	5.5	-			
25	11.6 12.9	11.9	10.4	10.0	10.3	9.6	9.3	10.1	11.7	9.9	10.0			

Sheep No.	Pre-infection	Weeks Post-infection									
		2	5	8	11	14	17	20	25	31	
14	13.2 13.0	11.7	11.3	12.2	12.8	11.3	10.3	12.0	11.7	12.3	
30	12.3 12.9	10.5	11.4	11.0	10.6	10.6	10.2	11.7*			
20	12.0 12.2	9.9	1	9.6	8.4	9.1	8.0	7.0	8.0		

Sheep No.	Pre-infection	Weeks Post-infection								
		3	6	9	12	15	18	20	25	30
17	10.1 11.2	10.0	8.6	9.3	7.7	5.6	4.3	3.4		
24	12.5 12.3	11.4	11.6	11.4	9.6	9.3	8.0	8.0*	9.2	-

Mean Corpuscular Volume (c.μ.)

Sheep No.	Pre-infection	Weeks Post-infection												
		1	4	7	10	13	16	19	23	27	31			
13	32.5 34.4	32.3	36.7	32.7	33.1	33.1	35.4	34.0	32.1	32.4	34.9			
23	34.1 29.2	35.1	36.7	36.5	34.0	34.5	37.9	36.2	34.9	34.5	36.2*			
25	32.3 32.5	33.7	37.3	35.6	35.3	33.7	36.1	29.7	35.9	35.3	36.6			

Sheep No.	Pre-infection	Weeks Post-infection									
		2	5	8	11	14	17	20	25	31	
14	32.6 30.3	33.6	33.6	32.8	33.3	32.6	34.3	32.0	33.2	33.4	
30	32.8 36.2	32.6	34.9	32.0	31.4	31.0	32.4	33.1*			
20	31.4 32.9	33.6	34.1	34.0	33.8	33.2	38.2	39.9	34.3	37.6*	

Sheep No.	Pre-infection	Weeks Post-infection								
		3	6	9	12	15	18	20	25	30
17	32.6 29.8	33.5	32.8	32.9	30.5	34.3	33.2	33.5		
24	31.4 36.5	34.8	35.2	38.0	35.2	32.6	35.0	34.7*	35.8	30.1

*The last record was at 30 weeks post-infection in the case of sheep 23 and 20, and at 21 weeks for No.30. That shown under week 20 for No.24 was done at week 21 post-infection.

Mean Corpuscular Haemoglobin (μμg)

Sheep No.	Pre-infection	Weeks Post-infection												
		1	4	7	10	13	16	19	23	27	31			
13	10.6 11.0	10.7	12.0	10.3	10.5	10.6	11.1	11.3	11.3	11.2	11.3			
23	12.4 9.3	11.1	11.2	10.7	10.1	10.9	11.0	9.8	10.1	10.5	-			
25	10.6 10.6	10.6	10.5	10.6	10.8	9.5	10.2	8.9	11.4	11.3	10.5			

Sheep No.	Pre-infection	Weeks Post-infection									
		2	5	8	11	14	17	20	25	31	
14	10.9 9.6	10.8	10.5	10.7	10.9	9.5	10.1	10.1	10.7	10.4	
30	10.0 11.4	10.1	11.4	10.3	9.2	9.4	9.4	11.4*			
20	10.2 10.3	10.2	11.1	10.5	10.0	11.2	11.8	10.4	10.6	-	

Sheep No.	Pre-infection	Weeks Post-infection								
		3	6	9	12	15	18	20	25	
17	10.3 10.0	10.5	9.7	10.6	9.8	10.1	9.9	10.4		
24	10.6 12.1	11.7	11.1	12.4	10.2	10.5	10.2	9.9*	11.6	

*The record in each case is for week 21 and not week 20 post-infection.

Mean Corpuscular Haemoglobin Concentration (%)

Sheep No.	Pre-infection	Weeks Post-infection												
		1	4	7	10	13	16	19	23	27	31			
13	32.4 31.9	33.2	32.8	31.6	31.7	31.9	31.4	33.3	35.1	34.6	32.2			
23	36.2 31.8	31.6	30.6	29.4	29.6	31.7	29.0	26.9	28.9	30.6	-			
25	32.7 32.6	31.3	28.1	29.8	30.7	28.3	28.2	30.1	31.6	31.9	28.6			

Sheep No.	Pre-infection	Weeks Post-infection									
		2	5	8	11	14	17	20	25	31	
14	33.4 31.7	32.0	31.4	32.5	32.8	29.0	29.4	31.6	32.0	31.1	
30	30.4 31.5	30.9	32.6	32.3	29.4	30.3	29.1	34.4*			
20	32.4 31.3	30.5	32.6	31.0	29.5	33.7	30.8	25.9	30.8	-	

Sheep No.	Pre-infection	Weeks Post-infection								
		3	6	9	12	15	18	20	25	
17	31.6 33.4	31.2	29.6	32.1	32.1	29.5	29.6	30.9		
24	33.8 33.2	33.5	31.3	32.6	29.1	32.1	29.1	28.6*	32.3	

Appendix Table 11A Live-weights (lb).

Infected Group

Sheep No.	Preinfection	Weeks Post-infection																									
		2	3	4	5	7	8	9	10	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
X190	66	67	66	69	70	71	72	73	72	70	67	70	70	70	72	73	72	75	70	76	77	80	79	81	81	77	79
X192	69	70	70	72	73	76	76	78	79	81	80	78	78	78	80	80	81	81	83	83	84	86	84	86	86	85	86
X196	70	70	69	71	72	73	75	78	76	79	79	77	77	77	78	78	78	79	80	80	81	83	82	84	85	81	85
X198	74	74	74	78	78	80	82	85	84	82	84	84	85	85	86	90	88	90	90	91	92	94	94	93	96	93	94
X204	62	67	67	68	68	70	72	75	75	75	77	71	74	75	75	78	78	78	80	81	80	83	84	86	85	83	84
X206	61	66	64	67	67	71	71	75	74	75	74	71	68	72	74	76	76	78	75	79	81	82	83	83	84	82	86
X211	68	70	70	70	72	78	76	79	80	81	81	80	78	80	81	81	83	85	83	85	85	86	85	87	86	85	86
X214	67	67	68	71	71	73	74	78	77	78	80	77	75	75	78	79	81	82	80	83	85	86	85	86	88	86	87
X215	67	67	66	69	70	73	72	76	77	76	78	74	76	76	78	77	78	80	80	81	81	85	84	85	86	83	85
X217	63	66	64	67	67	70	71	72	72	74	72	71	71	73	74	75	76	73	74	77	77	78	80	80	82	80	80

Uninfected Group

Sheep No.	Preinfection	Weeks Post-infection																									
		2	3	4	5	7	8	9	10	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
X189	70	72	72	74	75	77	79	81	81	84	84	83	85	85	87	88	90	91	91	92	92	96	95	96	99	94	97
X195	64	64	64	66	66	70	69	70	72	74	74	73	75	75	76	76	77	79	78	80	81	81	82	83	84	80	81
X197	69	67	73	75	75	78	79	81	81	81	81	82	83	83	84	87	86	88	90	90	91	94	94	94	95	91	94
X199	75	75	75	79	81	86	89	89	89	91	92	92	94	93	96	96	96	97	100	100	101	104	103	105	106	102	105
X201	65	67	68	69	71	74	75	77	78	79	83	81	82	83	84	84	84	85	85	87	89	91	91	90	91	88	91
X203	62	64	64	64	65	67	69	71	70	72	73	72	72	74	75	76	76	77	80	78	78	81	79	82	82	78	81
X207	64	65	64	68	68	72	71	72	71	75	76	76	74	76	76	76	76	76	75	80	81	82	80	82	82	80	83
X208	71	72	72	77	76	78	78	82	81	84	85	88	85	86	85	86	87	89	90	90	91	93	92	95	95	93	92
X209	62	65	63	65	65	67	70	72	71	72	74	74	74	75	75	77	79	78	79	80	80	81	82	84	85	80	84
X212	65	67	67	69	70	71	74	76	75	80	80	79	80	81	81	84	86	86	85	89	89	90	90	91	92	90	91

APPENDIX TABLE 11B CARCASS ANALYSIS

Sheep No.	Live Wt. Lbs.	Live Wt. Kilos	Warm Drsd.Carc. Kilos	24 hr. Cold Drsd.Carc. Kilos	Wt. of Spleen	(Corrected to 24 hr. C.C.W.). Wt. of Lean	Wt. of Fat	Wt. of Bone	Wt. of Kidneys	KO% on 24 C.D.W.	% Lean	% Fat	% Bone	% Kidney	Lean/ Bone Ratio	Within Exp. Carc. Rating	Within Exp. Score
INFECTED GROUP																	
X190	72	32.688	11.804	11.440	0.082	7.686	1.420	2.264	0.070	35.00	67.2	12.4	19.8	0.6	3.39	E	1
X192	80	36.320	14.528	14.425	0.075	9.417	2.342	2.590	0.076	39.72	65.3	16.2	18.0	0.5	3.64	B	4
X196	79	35.866	13.620	13.475	0.108	8.988	1.764	2.638	0.085	37.57	66.7	13.1	19.6	0.6	3.41	E	1
X198	88	39.952	15.890	15.640	0.092	10.154	2.536	2.874	0.076	39.15	64.9	16.2	18.4	0.5	3.53	B	4
X204	78	35.412	13.660	13.360	0.129	8.475	2.391	2.417	0.077	37.73	63.4	17.9	18.1	0.6	3.51	C	3
X206	78	35.412	14.560	14.250	0.085	9.608	2.046	2.522	0.074	40.24	67.4	14.4	17.7	0.5	3.81	C	3
X211	77	34.958	14.850	14.570	0.075	9.493	2.355	2.639	0.083	41.68	65.1	16.2	18.1	0.6	3.60	C	3
X214	80	36.320	14.820	14.550	0.096	9.584	2.378	2.494	0.094	44.51	66.0	16.3	17.1	0.6	3.84	D	2
X215	79	35.866	14.250	13.990	0.101	8.728	2.584	2.603	0.075	39.01	62.4	18.5	18.6	0.5	3.35	C	3
X217	75	34.050	13.540	13.270	0.087	8.197	2.598	2.408	0.067	38.97	61.8	19.6	18.1	0.5	3.40	C	3
TOTAL	786	356.844	141.522	138.970	0.930	90.330	22.414	25.449	0.777	39.358	650.2	160.8	183.5	5.5	35.48		27
UNINFECTED GROUP																	
X189	90	40.860	16.344	16.150	0.143	10.256	2.970	2.834	0.090	39.53	63.5	18.4	17.5	0.6	3.62	B	4
X195	75	34.050	13.166	12.950	0.080	7.660	2.902	2.314	0.074	38.03	59.2	22.4	17.9	0.6	3.31	C	3
X197	86	39.044	15.890	15.790	0.099	10.074	2.937	2.709	0.070	40.44	63.8	18.6	17.2	0.4	3.72	B	4
X199	100	45.400	19.522	19.100	0.104	10.715	5.392	2.907	0.086	42.07	56.1	28.2	15.2	0.5	3.69	A	5
X201	84	38.136	14.500	14.175	0.075	9.162	2.403	2.534	0.076	37.17	64.6	17.0	17.9	0.5	3.62	B	4
X203	75	34.050	13.840	13.520	0.090	8.280	2.843	2.315	0.082	39.71	61.3	21.0	17.1	0.6	3.58	B	4
X207	75	34.050	12.700	12.460	0.092	8.263	1.646	2.485	0.066	36.59	66.4	13.2	19.9	0.5	3.33	C	3
X208	86	39.044	14.425	14.100	0.083	9.630	1.446	2.940	0.084	36.11	68.2	10.3	20.9	0.6	3.28	D	2
X209	77	34.958	15.100	14.850	0.080	8.498	4.045	2.239	0.068	42.48	57.2	27.2	15.1	0.5	3.80	B	4
X212	85	38.590	16.250	15.940	0.079	9.432	3.900	2.532	0.076	41.31	59.1	24.5	15.9	0.5	3.73	B	4
TOTAL	833	378.182	151.737	149.035	0.925	91.970	30.484	25.809	0.772	39.344	619.3	200.8	174.6	5.3	35.68		37

C.C.W. = Cold carcass weight
K.O.% = Killing out per cent
C.D.W. = Cold dressed weight
Live-weight taken after all food with-held for 24 hours.
The carcasses were scored within the experiment as follows:
A - 5, B - 4, C - 3, D - 2, and E - 1.

Appendix Table 12A

Live-weights (lbs)

Calf No.	Group	Preinfection		Weeks post-infection															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
B449	2	216	228	240	258	272	277	298	304	315	323	325	326	321	321				
B451	1	241	247	262	271	283	286	304	311	321	330								
B452	2	276	282	298	308	317	320	333	340	346	361								
B453	C	213	220	236	251	263	267	282	289	310	312	314	326	330	352				
B454	1	311	323	334	350	361	363												
B456	1	232	244	261	281	288	294	322	320	324	333	365	372	384	372	384	391	397	404
B457	C	264	274	279	301	316	323	341	351	361	369								
B458	1	205	214	234	241	251	256	270	277	289	294	294	310	313	321				
B459	C	216	226	242	255	265	277	287	291	297	297	306	331	334	346	361	384	394	404
B460	2	289	298	307	329	342	344	351											
B461	C	291	297	317	335	347	350												
B502	2	238	243	256	272	284	284	299	306	317	323	336	340	329	302	300	278	271	

Appendix Table 12B

Serum Enzyme Assays

					Weeks post-infection															
O.C.T. (μ moles/ litre/min)	Calf No.	Group	Preinfection		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	B449	2	1.18	1.15	0.95	1.30	1.48	1.33	1.50	1.84	2.51	2.63	3.46	3.52	2.23	2.51				
	B451	1	1.39	1.50	1.58	1.71	1.64	1.68	1.50	1.53	1.35	1.70								
	B452	2	1.84	1.18	1.33	1.09	1.64	1.50	2.00	2.00	2.69	3.08								
	B453	C	1.04	1.22	1.19	0.99	1.18	0.86	0.75	0.86	0.89	0.96	0.83	0.93	0.60	0.89				
	B454	1	1.03	1.12	1.01	1.65	1.01	1.29												
	B456	1	1.35	1.38	1.58	1.15	1.18	1.29	0.89	0.60	2.0	1.82	1.79	2.43	3.78	3.70	2.51	2.88	2.72	3.29
	B457	C	1.33	1.38	1.35	1.18	0.92	1.22	1.07	1.12	1.15	1.22								
	B458	1	1.30	1.33	1.68	1.53	1.48	1.38	1.48	1.82	2.31	2.25	2.63	2.36	3.34	2.72				
	B459	C	1.47	1.29	1.33	0.98	0.92	1.30	1.09	1.07	0.89	1.18	1.01	1.33	0.46	1.32	1.79	1.07	0.80	0.67
	B460	2	1.25	0.95	1.42	1.64	1.76	2.08	1.71											
	B461	C	1.48	1.07	1.38	1.04	1.22	1.18												
	B502	2	1.50	1.50	1.29	1.48	1.12	1.50	1.82	2.0	2.72	3.29	3.38	4.30	2.66	1.50	1.29	1.45	0.92	
S.G.O.T. (μ moles/ litre/min)	B449	2	34.7	38.1	32.8	40.0	37.6	38.6	33.7	49.2	46.3	50.6	49.2	69.9	69.9	49.2				
	B451	1	56.9	81.9	77.1	53.0	60.3	46.3	44.3	60.3	56.9	60.3								
	B452	2	50.1	49.2	39.5	53.0	62.7	53.0	67.5	66.5	66.5	74.7								
	B453	C	26.5	28.0	25.1	25.1	35.2	25.5	25.1	25.5	33.7	24.1	21.2	26.5	21.2	21.2				
	B454	1	37.6	41.9	37.6	37.6	37.6	35.2												
	B456	1	45.8	53.0	49.6	43.4	40.5	46.3	49.2	50.6	52.5	62.2	45.8	79.5	94.0	84.4	52.5	46.3	43.9	52.5
	B457	C	41.9	43.4	24.1	33.7	37.6	35.2	34.7	35.2	35.2	35.2								
	B458	1	50.1	44.3	52.5	52.1	52.5	52.5	52.1	43.4	43.4	44.3	50.6	72.3	60.0	59.3				
	B459	C	50.1	53.0	49.6	40.0	56.9	43.9	35.2	39.5	43.4	39.5	38.6	45.8	44.3	40.0	38.1	35.2	38.1	43.4
	B460	2	45.8	53.0	43.4	38.1	43.4	43.9	54.9											
	B461	C	28.4	36.2	33.7	32.8	32.8	33.3												
	B502	2	26.5	28.4	32.8	32.8	32.8	36.2	32.8	32.8	38.6	43.9	43.9	49.2	45.8	30.4	21.7	22.7	21.2	
Alkaline Phosphatase (King-Armstrong units)	B449	2	-	18.5	10.0	14.2	16.7	16.5	13.1	15.2	14.6	15.8	16.5	16.4	-	15.7				
	B451	1	29.9	32.9	23.8	17.8	18.1	19.3	26.2	22.0	19.2	18.5								
	B452	2	13.6	16.2	10.3	14.2	13.9	15.1	15.4	16.2	8.9	12.0								
	B453	C	13.1	15.0	11.2	14.7	10.9	11.1	12.1	9.7	11.0	8.4	7.7	11.6	-	11.1				
	B454	1	13.1	14.3	16.2	14.2	14.1	12.7												
	B456	1	13.1	15.1	17.6	15.4	13.7	14.5	15.8	13.9	12.7	11.9	9.1	11.3	11.5	18.2	14.4	12.1	12.8	15.3
	B457	C	15.7	18.3	10.0	15.4	16.5	18.0	14.0	13.8	11.2	13.2								
	B458	1	15.6	15.4	16.1	11.4	12.5	13.2	15.4	16.4	9.8	9.6	9.0	11.4	-	11.9				
	B459	C	12.2	15.3	21.1	15.6	15.6	14.1	13.4	14.2	13.7	11.2	10.1	14.4	18.5	15.0	13.5	14.1	15.9	-
	B460	2	17.6	14.4	13.1	14.3	14.3	12.7	16.7											
	B461	C	10.3	11.5	11.2	11.3	11.6	9.0												
	B502	2	7.9	8.6	6.5	6.3	6.5	8.6	7.9	8.2	7.0	7.9	8.9	8.4	8.4	5.7	5.7	4.4	5.3	
Sorbitol dehydrogenase (μ moles/ litre/min)	B449	2	3.6	2.3	3.4	-	2.8	-	3.8	-	7.8	-	9.1	-	7.5	0				
	B453	C	1.0	1.3	0.3	-	0.3	-	1.3	-	1.6	-	2.0	-	1.0	0				
	B456	1	-	2.7	3.8	1.9	-	2.3	-	1.9	0.6	-	-	6.7	3.0	-	0	-	-	-
	B458	1	2.2	0.3	-	0.4	2.2	-	3.4	3.4	14.4	-	-	-	8.3	10.7				
	B459	C	-	0.4	1.9	1.2	-	0	0.8	-	-	1.7	0.9	0.9	-	-	2.0	-	0.7	-
	B502	2	1.0	1.5	-	1.7	-	1.0	-	4.6	-	5.7	4.0	-	0.7	-	0.6	-	-	

Appendix Table 12C

Total Bilirubin (mg/100 ml)

Calf No.	Group	Preinfection		Weeks post-infection										
				2	4	6	8	10	11	12	13	14	15	16
B449	2	0.06	0.09	0.04	0.09	0	0.12	0.04		0.05				
B451	1	0.19	0.16	0.12	0.03	0.16	0.12							
B452	2	0.09	0.07	0.07	0	0.09	0.13							
B453	C	0.20	0.12	0.14	0.08	0.04	0.12	0.08		0.05				
B454	1	0.12	0.04	0.07	0									
B456	1	0.13	0.05	0.08	0.21	0	0	0.03		0.21		0.25	0.26	0.12
B457	C	0.09	0.05	0.12	0.05	0.07	0.09							
B458	1	0.15	0.14	0.08	0.01	0.08	0.03	0.03		0.12				
B459	C	0.15	0.13	0.07	0	0.03	0.07	0.01		0.09		0.01		0.12
B460	2	0.09	0.08	0.08	0.08									
B461	C	0.10	0.08	0.01	0.16									
B502	2	0.14	0.14	0.12	0.12	0.03	0.16	0.07	0.23	1.50	0.84	2.94	6.48	

Protein-bound hexose (mg/100 ml)

Calf No.	Group	Preinfection		Weeks post-infection										
				1	3	5	7	9	11	12	13	15	16	
B449	2	104	104	115	101	106	117	120	134	156				
B453	C	95	94	94	106	99	111	108	124	120				
B456	1	106	106	107	111	101	116	124	138		163	183	156	
B458	1	93	98	100	100	104	116	115	137	151				
B459	C	111	99	109	106	96	108	94	113		107	91	99	
B502	2	109	93	96	101	93	113	111	145		173	174		

Appendix Table 12D

Total and fractional serum protein concentrations

(G/100 ml)

Calf No.	Group	Fraction		Preinfection		Weeks post-infection									
						1	3	5	7	9	11	12	13	15	16
B458	1	Albumin		2.2	2.2	2.4	2.3	2.0	2.0	1.9	1.9	1.6			
		Alpha	Globulin	1.5	1.5	1.3	1.3	1.5	1.4	1.4	1.8	1.9			
		Beta	Globulin	1.0	1.3	1.0	0.9	0.9	1.2	0.8	0.9	1.3			
		Gamma	Globulin	1.5	1.9	1.7	1.5	1.7	1.3	1.6	1.9	2.4			
		Total		6.2	6.8	6.3	6.0	6.1	5.9	5.7	6.5	7.2			
B456	1	Albumin		2.4	2.5	2.6	2.2	2.1	1.8	1.9	1.9	-	1.8	2.4	2.0
		Alpha	Globulin	1.3	1.2	1.2	1.3	1.3	1.5	1.3	1.6	-	2.0	2.4	2.1
		Beta	Globulin	0.8	0.7	0.7	0.7	0.7	0.8	0.7	0.8	-	1.1	1.1	1.1
		Gamma	Globulin	2.0	1.7	1.7	1.9	1.7	1.7	1.7	2.0	-	2.5	3.3	2.7
		Total		6.5	6.1	6.2	6.1	5.8	5.8	5.7	6.3	-	7.4	9.2	7.9
B449	2	Albumin		2.4	2.2	2.2	2.1	2.2	1.9	1.9	1.8	2.0			
		Alpha	Globulin	1.2	1.4	1.3	1.1	1.2	1.3	1.5	1.7	2.1			
		Beta	Globulin	0.9	0.8	0.8	0.8	0.9	1.1	0.9	1.0	0.9			
		Gamma	Globulin	1.9	1.8	1.8	1.8	1.8	2.0	1.8	2.0	2.5			
		Total		6.5	6.3	6.2	5.8	6.1	6.4	6.1	6.4	7.5			
B502	2	Albumin		2.3	2.1	2.2	2.0	2.3	1.9	1.9	1.9	-	1.9	1.5	
		Alpha	Globulin	1.4	1.6	1.6	1.5	1.4	1.6	1.7	1.6	-	2.2	1.5	
		Beta	Globulin	0.8	0.9	0.8	0.6	0.7	0.8	0.8	0.9	-	0.9	1.1	
		Gamma	Globulin	2.2	2.2	2.4	2.2	1.9	2.1	2.1	2.7	-	2.8	2.6	
		Total		6.7	6.9	7.0	6.3	6.3	6.5	6.5	7.1	-	7.9	6.7	
B453	C	Albumin		2.3	2.1	2.1	1.9	2.2	2.0	1.9	2.0	2.2			
		Alpha	Globulin	1.5	1.3	1.3	1.3	1.2	1.5	1.5	1.5	1.3			
		Beta	Globulin	1.0	0.9	0.7	0.9	0.7	0.7	0.7	0.9	0.8			
		Gamma	Globulin	2.2	1.7	2.3	1.9	1.6	1.8	1.9	1.7	1.8			
		Total		6.9	5.9	6.4	6.0	5.8	6.0	6.0	6.1	6.1			
B459	C	Albumin		2.1	2.4	2.2	-	1.8	-	1.8	1.8	-	2.0	-	2.4
		Alpha	Globulin	1.5	1.3	1.4	-	1.2	-	1.3	1.4	-	1.2	-	1.2
		Beta	Globulin	1.1	0.8	1.0	-	1.2	-	0.7	0.8	-	0.9	-	1.0
		Gamma	Globulin	1.8	1.8	1.9	-	1.6	-	1.5	1.2	-	1.6	-	1.9
		Total		6.5	6.3	6.6	5.9	5.8	5.8	5.4	5.3	-	5.7	6.3	6.5

Appendix Table 12E

Haematological results, leucocyte series

Calf No.		Preinfection			Weeks post-infection															
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
B449	Neutrophils (band)	68	0	0	0	0	0	0	0	0	0	0	54	0	0	0				
	Neutrophils (segmented)	1302	2176	2162	2048	3404	1365	2405	3596	3162	2562	2368	1790	978	1592	3540				
	Eosinophils	206	0	141	341	888	945	573	812	920	1647	1700	2929	2357	4848	5236				
	Basophils	274	256	141	49	444	158	228	0	173	183	52	163	173	0	154				
	Lymphocytes	11234	9664	6674	6874	9768	7822	7786	6960	6670	7442	5665	5588	7590	6925	5852				
	Monocytes	616	704	282	438	296	210	458	232	575	366	515	326	402	485	618				
	Total leucocytes	13700	12800	9400	9750	14800	10500	11450	11600	11500	12200	10300	10850	11500	13850	15400				
B451	Neutrophils (band)	0	0	0	0	0	0	0	0	0	0	0								
	Neutrophils (segmented)	2951	2952	1502	1418	1486	2138	1106	1750	2031	2182	1764								
	Eosinophils	638	504	230	645	2264	1639	2729	2170	1057	1355	1544								
	Basophils	239	288	116	0	70	0	74	210	0	75	0								
	Lymphocytes	11484	10152	9413	10256	9764	9975	10398	9310	12430	13761	13952								
	Monocytes	638	504	289	581	566	498	443	560	732	677	440								
	Total leucocytes	15950	14400	11550	12900	14150	14250	14750	14000	16250	15050	14700								
B452	Neutrophils (band)	0	0	0	0	0	0	0	0	0	0	0								
	Neutrophils (segmented)	1829	2640	2228	2277	1854	1934	584	1708	3876	1538	1998								
	Eosinophils	261	180	248	446	721	1050	511	915	1224	820	1242								
	Basophils	52	240	149	98	0	55	0	61	68	154	108								
	Lymphocytes	7890	8280	6730	6633	7313	7569	5840	8906	7344	7431	6750								
	Monocytes	418	660	545	446	412	442	365	610	1088	308	702								
	Total leucocytes	10450	12000	9900	9900	10300	11050	7300	12200	13600	10250	10800								
B453	Neutrophils (band)	0	29	0	0	0	36	0	85	39	0	0	0	94	51	0				
	Neutrophils (segmented)	1298	1653	1978	803	2483	2308	824	2070	2184	2129	984	1208	2679	3384	1580				
	Eosinophils	86	27	86	149	286	140	52	42	0	209	205	264	0	51	41				
	Basophils	130	86	43	119	0	36	0	0	39	42	82	0	0	0	41				
	Lymphocytes	6574	3620	5934	4492	6208	3976	3708	5577	5187	5344	6519	5587	6157	5909	6116				
	Monocytes	562	285	559	387	573	604	566	676	351	626	410	491	470	705	322				
	Total leucocytes	8650	5700	8600	5950	9550	7100	5150	8450	7800	8350	8200	7550	9400	10100	8100				
B454	Neutrophils (band)	0	0	0	0	0	0	0												
	Neutrophils (segmented)	1134	2422	1945	1504	1790	1043	955												
	Eosinophils	63	0	115	558	348	783	573												
	Basophils	126	128	115	112	100	218	48												
	Lymphocytes	10521	9562	8187	8474	7463	6308	7640												
	Monocytes	756	638	1088	502	249	348	334												
	Total leucocytes	12600	12750	11450	11150	9950	8700	9550												
B456	Neutrophils (band)	68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	84	65
	Neutrophils (segmented)	1365	3432	2728	2280	2914	3885	2220	1840	2450	2480	2176	1185	2008	2340	2059	2672	2936	1837	1370
	Eosinophils	0	0	54	180	324	388	300	329	700	783	816	237	1108	1105	2411	3590	3578	2672	2414
	Basophils	273	65	107	60	194	65	120	66	70	0	68	59	138	65	213	167	92	84	65
	Lymphocytes	11057	9259	7383	8760	9259	8288	8640	10323	9870	9069	9656	9658	9834	8840	9017	9603	11102	11022	8483
	Monocytes	887	194	428	720	259	324	720	592	910	718	884	711	762	650	497	668	642	1001	653
	Total leucocytes	13650	12950	10700	12000	12950	12950	12000	13150	14000	13050	13600	11850	13850	13000	14200	16700	18350	16700	13050
B457	Neutrophils (band)	0	55	0	0	0	0	0	0	0	0	0								
	Neutrophils (segmented)	698	2671	1786	910	3843	1868	1598	729	2516	2021	619								
	Eosinophils	133	162	241	54	63	241	188	182	1564	1097	393								
	Basophils	0	109	0	0	0	60	0	0	0	0	0								
	Lymphocytes	5287	7303	6996	8933	8442	8676	6674	10631	8636	7854	9338								
	Monocytes	532	600	627	803	252	1205	940	608	884	578	900								
	Total leucocytes	6650	10900	9650	10700	12600	12050	9400	12150	13600	11550	11250								
B458	Neutrophils (band)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
	Neutrophils (segmented)	1384	1937	1817	2380	2081	1043	2100	1540	2498	2500	1328	1435	1832	1896	2108				
	Eosinophils	101	0	260	105	740	447	630	704	971	950	483	1394	944	1694	2652				
	Basophils	34	0	87	70	0	37	126	88	46	50	0	41	54	0	0				
	Lymphocytes	4725	7040	5707	4235	6059	5364	4914	6160	5180	6100	5595	4961	7881	9350	8296				
	Monocytes	506	473	779	210	370	559	630	308	555	400	644	369	389	610	544				
	Total leucocytes	6750	9450	8650	7000	9250	7450	8400	8800	9250	10000	8050	8200	11100	13550	13600				
B459	Neutrophils (band)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Neutrophils (segmented)	1584	3040	2940	4013	2195	1825	2160	2401	3675	5338	2902	2772	1932	2496	1892	1794	1580	2064	2256
	Eosinophils	45	0	98	0	77	42	0	196	262	1754	1297	420	368	160	352	131	158	430	376
	Basophils	45	96	294	214	116	83	324	98	105	227	62	42	138	0	0	0	79	215	47
	Lymphocytes	6426	6176	6223	5885	4928	5852	7722	6468	5880	7473	7657	4788	6164	4911	5940	6256	5648	5547	6439
	Monocytes	950	338	245	588	386	498	594	637	578	458	432	378	598	483	616	569	435	344	282
	Total leucocytes	9050	9650	9800	10700	7700	8300	10800	9800	10500	15250	12350	8400	9200	8050	8800	8750	7900	8600	9400
B460	Neutrophils (band)	0	0	0	0	0	0	0	0											
	Neutrophils (segmented)	798	1496	1846	1522	2115	1750	1775	2672											
	Eosinophils	0	0	211	87	646	1820	2130	1662											
	Basophils	76	48	0	0	0	70	142	145											
	Lymphocytes	6384	7624	7754	6525	8695	9800	9372	9465											
	Monocytes	342	482	739	566	294	560	781	506											
	Total leucocytes	7600	9650	10550	8700	11750	14000	14200	14450											
B461	Neutrophils (band)	0	0	0	0	0	0	0												
	Neutrophils (segmented)	1050	942	1770	2137	1700	3022	2004												
	Eosinophils	150	0	220	65	52	78	0												
	Basophils	40	135	74	0	52	78	0												
	Lymphocytes	7550	11768	12243	10230	8188	11548	8874												
	Monocytes	1200	605	443	518	308	774	572												
	Total leucocytes	10000	13450	14750	12950	10300	15500	11450												
B502	Neutrophils (band)	0	0	0	0	0	0	0	62	0	0	0	0	0	110	1140	190	403	767	
	Neutrophils (segmented)	2825	1979	2858	2943	2563	1638	2418	1660	2363	2619	2184	1265	2415	2585	10394	6935	8260	8695	
	Eosinophils	134	102	63	490	937	526	682	492	1630	1237	819	964	2001	1155	4183	4465	2519	426	
	Basophils	67	203	0	164	125	0	186	62	0	0	68	60	69	0	0	95	101	0	
	Lymphocytes	10222	7308	9144	7085	8375	9185	8680	9778	11899	10476	10306	9519	8556	6435	9126	7030	8363	6309	
	Monocytes	202	558	635	218	500	351	434	246	408	218	273	242	759	715	507	285	504	853	
	Total leucocytes	13450	10150	12700	10900	12500	11700	12400	12300	16300	14550	13650	12050	13800	11000	25350	19000	20150	17050	

All results expressed in numbers of cells per cubic millimetre of blood.

Appendix Table 12F

Haematological results, erythrocyte series

	Calf No.	Group	Preinfection			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Packed Cell Volume (%)	B449	2	27½	29	28½	28½	28½	29	28	29	29½	30	30½	31½	33	32½	33				
	B451	1	33	32	35	36½	35	35	36	38	34½	34	35								
	B452	2	32½	31½	31	32	29½	32	29½	30½	31	30½	32½								
	B453	C	31½	31½	31	32½	32	32	34	35	33½	35	35	35	32	30½	30				
	B454	1	30½	31	28½	29½	28½	29	30												
	B456	1	33½	32½	32	32½	32½	33½	32	31½	32½	32½	35	33	33½	33½	35½	37	38½	40	37½
	B457	C	29½	31	30	29½	30	30½	31½	33½	33½	33½	30								
	B458	1	32½	35½	33	35	34½	34	34	35	34½	35	36½	33½	34	35½	35½				
	B459	C	28½	31	29	28½	29½	30	29½	30	31½	32½	35½	30½	29	31	28	27	28½	27	29½
	B460	2	29½	32	30½	30	30½	31	31½	32½											
	B461	C	29½	30½	31½	31	31½	34	30½												
	B502	2	30	29½	32½	32½	33½	33½	36	33½	33	33½	33½	33	32½	30½	26½	25½	21½	18½	
Red Cells (million/ cmm.)	B449	2	7.34	7.96	7.41	7.70	7.81	7.85	8.14	8.94	8.74	7.96	8.54	8.14	9.34	8.96	8.40				
	B451	1	7.53	7.55	7.68	7.93	7.89	8.24	8.52	9.33	8.41	8.71	8.65								
	B452	2	7.83	7.51	7.74	7.41	6.63	6.71	7.04	7.13	6.86	6.98	7.31								
	B453	C	7.81	8.19	8.06	8.19	8.06	8.34	9.08	9.20	8.80	9.06	9.53	8.69	8.87	8.78	8.68				
	B454	1	7.46	7.86	7.37	7.29	6.84	7.23	7.29												
	B456	1	7.60	8.06	7.19	7.28	7.63	7.47	7.71	7.53	7.81	7.68	7.82	7.69	7.69	7.79	8.08	7.41	7.54	7.69	6.91
	B457	C	7.02	7.51	7.18	6.92	6.67	7.40	7.32	8.01	7.44	7.74	6.98								
	B458	1	8.95	9.47	7.75	8.91	9.23	9.06	8.34	9.85	8.92	9.72	9.48	9.17	8.76	9.65	8.82				
	B459	C	6.28	7.03	5.86	7.16	6.77	6.91	6.49	7.28	6.91	7.44	8.88	7.43	7.14	7.72	7.34	6.26	6.39	6.49	6.48
	B460	2	6.96	7.23	6.86	6.95	6.82	7.63	7.29	8.23											
	B461	C	6.58	6.61	7.08	7.37	6.95	7.36	6.76												
	B502	2	7.16	7.66	8.08	8.63	9.18	8.51	8.89	9.09	9.58	8.93	8.88	8.18	7.84	7.89	7.13	5.55	3.94	3.54	
Haemoglobin (G./100 ml.)	B449	2	9.1	10.0	9.3	9.0	9.3	9.7	9.3	9.7	9.7	9.7	10.0	10.7	10.9	10.8	10.9				
	B451	1	11.3	11.0	11.1	11.6	11.4	11.7	11.5	11.9	11.4	11.3	11.6								
	B452	2	10.4	10.1	9.6	10.0	9.3	9.7	9.3	9.5	9.9	9.7	10.3								
	B453	C	10.5	11.4	9.9	10.1	10.4	10.0	10.7	11.7	11.1	10.8	11.1	11.4	10.4	9.8	10.0				
	B454	1	10.2	10.5	9.1	9.4	9.0	9.0	10.0												
	B456	1	11.0	10.7	10.4	10.5	10.5	10.8	10.5	10.1	10.5	10.7	11.3	10.8	11.4	10.6	11.4	12.1	12.4	13.1	12.7
	B457	C	9.7	10.0	9.3	9.0	9.5	9.3	9.8	10.3	10.6	10.5	9.3								
	B458	1	9.8	11.9	10.4	10.5	11.1	10.8	10.7	11.2	11.1	11.4	10.7	11.2	11.5	11.4	11.5				
	B459	C	9.8	10.0	9.1	9.4	9.3	9.1	9.4	9.5	9.5	10.3	11.1	9.9	9.7	10.2	9.0	8.9	9.0	8.7	9.6
	B460	2	10.1	10.9	9.5	9.7	9.7	9.8	10.4	10.3											
	B461	C	10.0	10.5	10.1	10.2	10.1	10.8	10.1												
	B502	2	10.1	10.0	10.2	10.2	11.4	10.5	11.2	10.7	10.5	10.6	10.9	10.9	10.8	9.8	8.4	8.0	6.4	5.4	
Mean Corpuscular volume (c.μ.)	B449	2	37.5	36.4	38.5	37.0	36.5	36.9	34.4	32.4	34.0	37.7	35.7	38.7	35.3	36.3	39.3				
	B451	1	43.8	42.4	45.6	46.0	44.3	42.4	42.3	40.7	41.0	39.0	40.5								
	B452	2	41.5	41.9	40.0	43.2	44.6	47.7	41.9	42.8	45.2	43.7	44.4								
	B453	C	40.3	38.4	38.4	39.7	39.7	38.4	37.5	38.0	38.1	38.6	36.7	40.3	36.1	34.7	34.5				
	B454	1	40.9	39.4	38.7	40.4	41.6	40.1	41.2												
	B456	1	44.1	40.3	44.5	44.7	42.6	44.8	41.5	41.8	41.6	42.3	44.8	42.9	43.5	43.0	43.9	49.9	51.0	52.0	54.3
	B457	C	42.0	41.3	41.8	42.2	45.0	41.2	43.0	41.8	45.0	43.2	43.0								
	B458	1	36.3	37.5	42.6	39.3	37.4	37.5	40.7	35.5	38.7	36.0	38.2	36.5	38.8	36.8	40.3				
	B459	C	45.4	44.1	49.5	39.8	43.6	43.4	45.5	41.2	45.6	43.6	40.3	41.1	40.6	40.2	38.2	43.2	44.6	41.6	45.5
	B460	2	42.4	44.3	44.4	43.2	44.7	40.6	43.2	39.5											
	B461	C	44.5	46.1	44.5	42.1	45.3	46.2	45.1												
	B502	2	41.9	38.5	40.2	37.6	36.5	39.3	40.5	37.1	34.4	37.5	38.0	40.4	41.4	38.7	37.2	46.0	54.6	52.2	
Mean Corpuscular Haemoglobin (μμg.)	B449	2	12.4	12.5	12.6	11.7	12.0	12.3	11.4	10.8	11.2	12.1	11.7	13.1	11.6	12.1	13.0				
	B451	1	14.9	14.5	14.5	14.7	14.5	14.1	13.5	12.7	13.6	13.0	13.4								
	B452	2	13.3	13.4	12.4	13.5	14.1	14.4	13.2	13.4	14.4	13.8	14.1								
	B453	C	13.5	13.9	12.2	12.4	12.9	12.0	11.8	12.8	12.7	11.9	11.7	13.1	11.7	11.2	11.5				
	B454	1	13.7	13.4	12.4	12.9	13.1	12.4	13.7												
	B456	1	14.4	13.2	14.4	14.5	13.8	14.5	13.7	13.4	13.4	13.9	14.4	14.0	14.8	13.6	14.2	16.3	16.5	17.0	18.4
	B457	C	13.8	13.4	13.0	13.0	14.2	12.5	13.4	12.9	14.2	13.5	13.4								
	B458	1	11.0	12.6	13.5	11.8	12.1	11.9	12.8	11.3	12.5	11.8	11.3	12.3	13.1	11.8	13.0				
	B459	C	15.7	14.3	15.6	13.2	13.7	13.2	14.5	13.1	13.8	13.8	12.6	13.4	13.6	13.2	12.2	14.2	14.1	13.4	14.8
	B460	2	14.6	15.1	13.9	13.9	14.2	12.9	14.2	12.5											
	B461	C	15.2	15.8	14.3	13.9	14.5	14.7	14.9												
	B502	2	14.1	13.1	12.7	11.9	12.4	12.4	12.6	11.7	10.8	11.9	12.2	13.3	13.8	12.4	11.7	14.4	16.3	15.3	
Mean Corpuscular Haemoglobin Concentration (%)	B449	2	33.0	34.3	32.7	31.6	32.7	33.4	33.1	33.3	32.7	32.2	32.8	33.9	32.9	33.2	33.1				
	B451	1	34.1	34.2	31.8	31.8	32.6	33.3	31.9	31.2	33.1	33.3	33.0								
	B452	2	31.9	32.1	31.0	31.2	31.6	30.2	31.6	31.2	31.8	31.6	31.8								
	B453	C	33.4	36.1	31.8	31.1	32.6	31.1	31.4	33.5	33.2	30.7	31.8	32.4	32.4	32.2	33.2				
	B454	1	33.6	34.0	32.0	31.9	31.5	31.0	33.2												
	B456	1	32.7	32.8	32.4	32.4	32.4	32.2	32.9	32.1	32.2	32.9	32.2	32.6	33.9	31.7	32.2	32.6	32.3	32.6	33.9
	B457	C	32.8	32.3	31.1	30.6	31.5	30.4	31.2	30.9	31.6	31.3	31.1								
	B458	1	30.3	33.6	31.6	30.1	32.3	31.8	31.4	31.9	32.3	32.7	29.5	33.5	33.8	32.0	32.4				
	B459	C	34.5	32.3	31.4	33.0	31.4	30.4	31.9	31.8	30.2	31.7	31.2	32.6	33.4	32.9	32.0	32.8	31.5	32.2	32.4
	B460	2	34.3	34.1	31.3	32.3	31.7	31.7	32.9	31.7											
	B461	C	34.1	34.3	32.1	33.0	32.1	31.8	33.1												
	B502	2	33.8	34.0	31.5	31.5	33.9	31.4	31.2	31.6	31.7	31.6	32.1	33.0	33.2	32.1	31.5	31.4	29.9	29.3	

Appendix Table 13A Live-weights (lbs.)

Calf No.	Group	Preinfection		Weeks Post-infection														
				1	3	5	6	7	8	9	10*	11	12	13	14	15	16	18
B498	2	409	418	432	451	465	467	485	481	488	478	491	500	490	503	508	512	514
B499	3	428	438	434	469	479	479	493	495	504	508	506	514	516	526	533	537	542
B500	2	425	427	443	467	484	479	495	499	511	510	514	512	515	517	537	543	552
B501	2	437	445	459	469	484	480	500	508	519	517	517	511	508	515	515	516	512
B503	3	441	459	467	481	500	496	514	518	536	536	541	543	544	547	562	566	571
B504	2	462	472	476	501	520	514	537	527	541	536	544	546	548	547	557	559	555
B505	3	389	400	410	422	435	431	452	445	460	465	469	471	476	491	495	501	493
B506	1	423	434	450	462	481	478	488	492	485	484	492	505	504	499	511	512	513
B507	C	499	507	514	534	545	544	561	570	585	584	586	594	598	590	611	612	619
B509	1	461	470	483	505	521	521	530	540	542	536	539	547	548	559	567	571	559
B510	3	440	454	471	493	495	506	523	526	537	535	537	545	554	552	562	566	567
B511	C	390	400	414	433	452	452	465	467	468	471	474	486	484	493	505	516	513
B512	1	404	409	420	429	448	442	464	467	477	475	477	483	479	484	490	496	497
B513	1	445	449	474	491	509	508	530	532	538	543	547	544	553	559	576	573	574

*Time of infection of Group 3

Calf No.	Group	Preinfection		Weeks Post-infection						
				1	2	3	4	5	6	8
B447	4	565	574	586	594	596	585	609	622	600
B448	4	507	518	514	510	518	533	560	563	562
B875	4	637	648	646	636	659	657	675	688	685
B968	4	553	550	543	550	553	543	550	555	561
C28	C	546	559	548	567	599	594	580	593	610
C37	C	511	-	-	525	533	547	573	567	580

Appendix Table 13B

Biochemical Assays on Sera

	Group	Calf No.	Preinfection	Weeks Post-infection										
				0	2	4	6	8	9	11	12	14	16	18/19
Alkaline Phosphatase (King-Armstrong Units)	2	B498	10.9	10.0	10.9	14.6	15.1	18.0	14.7	11.5	11.8	15.2	13.7	17.8
		B500	11.2	10.0	9.7	12.6	13.1	11.6	13.5	15.0	15.4	18.2	15.2	13.5
		B501	8.5	11.0	10.4	13.6	11.4	11.6	10.5	7.8	9.3	12.6	15.1	15.2
		B504	9.5	9.5	11.1	12.1	11.7	11.3	11.4	13.1	20.8	11.1	17.7	11.4
	1	B506	8.0	8.0	-	9.2	10.7	9.5	5.3	8.0	7.7	10.2	12.0	10.4
		B509	9.0	11.3	10.6	11.2	10.0	7.8	9.5	8.4	9.5	15.9	9.7	8.3
		B512	9.3	7.3	9.7	10.2	11.2	11.8	12.0	11.3	10.9	12.1	11.0	7.3
		B513	9.3	12.1	12.7	13.9	13.9	13.2	12.2	10.4	13.5	13.2	13.7	13.1
	C	B507	5.1	3.8	3.8	6.8	5.3	7.6	5.9	6.0	4.7	5.2	6.1	5.8
		B511	11.9	12.1	14.0	14.0	11.7	15.1	10.0	12.5	11.6	12.4	14.2	12.7
O.C.T. (μ moles/litre/min.)	2	B498	1.09	1.29	1.56	1.45	0.98	1.30	1.19	1.50	1.65	2.77	0.86	1.30
		B500	0.23	0.18	0.81	1.04	1.18	1.12	2.25	2.25	3.60	3.29	1.79	2.00
		B501	0.50	1.38	0.58	0.60	1.79	1.64	2.20	2.51	3.55	5.08	3.60	2.59
		B504	1.29	0.96	0.80	0.93	1.15	0.98	1.50	1.07	1.64	2.74	2.40	1.53
	1	B506	1.15	0.86	0.38	1.41	0.31	0.41	1.38	1.18	1.45	3.38	1.42	1.90
		B509	0.95	0.92	1.01	0.70	0.61	0.60	1.45	1.29	2.28	2.80	1.71	1.35
		B512	0.96	1.35	1.04	0.49	0.60	1.61	1.22	1.22	0.95	2.72	2.57	0.75
		B513	1.07	1.10	0.93	0.57	0.26	1.45	1.16	1.27	2.19	1.79	1.87	0.78
	C	B507	0.60	0.81	0.67	0.18	0.83	0.38	0.92	0.43	0.92	0.96	0.78	0.70
		B511	0.43	1.27	1.22	1.30	0.52	1.65	1.38	0.44	0.34	1.01	0.55	0.78
S.G.O.T. (μ moles/litre/min.)	2	B498	53.0	49.2	40.0	69.9	66.5	56.9	60.3	55.9	49.2	54.0	49.2	30.8
		B500	53.5	61.2	60.3	60.3	74.7	115.6	106.0	110.9	130.1	106.0	86.8	67.5
		B501	67.5	62.7	60.3	73.3	81.9	81.9	96.4	86.8	106.0	96.4	106.0	62.7
		B504	43.4	38.1	46.3	40.0	46.3	43.4	49.2	49.2	55.9	54.0	77.1	53.0
	1	B506	40.0	49.2	43.4	56.9	53.5	49.2	74.7	77.1	81.9	86.8	81.9	62.7
		B509	49.2	49.2	60.3	56.9	62.7	54.5	86.8	86.8	106.0	86.8	77.1	57.8
		B512	53.0	49.2	43.4	78.6	60.3	69.9	69.9	92.1	86.8	101.2	96.4	53.0
		B513	53.0	60.3	56.9	74.7	46.3	81.9	74.7	106.0	135.0	74.7	68.4	64.1
	C	B507	35.2	46.3	36.2	53.0	45.3	46.3	46.3	45.8	45.3	39.5	46.8	35.7
		B511	78.6	69.9	66.5	81.9	79.5	74.7	56.9	55.9	61.2	66.0	73.3	51.6
Protein-Bound Hexose (mg/100 ml. serum)	2	B498	99	103	119	102	99	108	110	121	120	137	146	145
	2	B500	95	108	103	99	95	105	107	111	106	139	118	119
	1	B506	106	101	108	104	113	113	113	119	115	129	143	143
	1	B509	108	105	107	110	101	119	120	120	121	136	134	130
	C	B507	103	102	108	103	100	95	97	95	92	110	106	113
	C	B511	99	81	114	99	92	100	112	100	105	99	106	97

	Group	Calf No.	Preinfection		Weeks Post-infection																	
					0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	18	19
Sorbitol Dehydrogenase (μ moles/litre/min.)	2	B498	-	1.4	4.6	-	0	-	3.1	-	5.6	-	4.4	-	-	4.7	3.2	-	1.2	12.8	14.1	0	
		B500	-	-	1.7	-	1.6	-	1.8	-	3.2	3.6	-	3.3	-	5.7	4.9	7.2	-	5.2	2.8	2.2	
		B501	-	1.5	3.3	-	4.1	-	3.1	-	5.0	-	5.6	3.4	-	6.3	-	13.1	-	12.2	6.9	3.9	
		B504	-	0.9	2.2	-	0.9	-	1.9	-	0.9	-	3.0	3.8	5.6	-	3.3	-	3.1	6.3	7.1	-	2.3
	1	B506	1.4	3.4	-	-	-	2.4	-	-	-	3.5	-	-	-	3.4	-	2.5	2.1	4.4	2.1	3.9	
	C	B507	-	1.7	1.0	-	2.3	1.9	-	-	-	2.2	1.2	-	-	1.5	0	-	0	2.0	1.3	0	
	3	B499	0	1.8	-	0	3.6	-	1.5	3.9	-	5.4	4.6										
		B503	3.8	3.6	-	2.7	1.8	-	2.4	4.7	-	4.4	2.8										
		B505	2.7	1.2	-	1.3	0	-	1.8	2.1	-	4.3	2.9										

Appendix Table 13C

Total and Fractional Serum Protein Concentrations (g./100 ml.)

Calf No.	Group No.	Fraction		Preinfection	Weeks Post-infection										
					0	2	4	6	8	9	11	12	14	16	18/19
B506	1	Albumin		2.2	2.1	1.6	1.7	1.7	2.0	2.0	1.9	1.8	2.1	1.9	2.0
		Alpha	Globulin	1.4	1.3	1.5	1.3	1.2	1.4	1.4	1.4	1.6	1.7	1.9	1.8
		Beta	Globulin	0.9	0.7	0.8	1.0	0.9	0.8	0.9	0.9	1.0	0.9	1.1	1.2
		Gamma	Globulin	2.2	2.4	2.2	2.7	3.0	2.5	2.4	2.9	2.9	2.8	3.5	3.3
		Total		6.5	6.4	6.1	6.7	6.7	6.8	6.6	7.0	7.3	7.6	8.4	8.4
B509	1	Albumin		1.7	1.8	1.9	1.9	1.5	-	2.0	2.3	1.8	1.2	2.0	1.8
		Alpha	Globulin	1.4	1.5	1.4	1.5	1.5	-	1.6	1.5	1.6	2.2	1.8	1.7
		Beta	Globulin	1.0	0.9	1.0	1.0	0.9	-	1.0	1.0	0.9	1.2	1.1	1.1
		Gamma	Globulin	2.5	2.7	2.4	2.4	2.4	-	2.6	2.9	3.1	3.3	3.4	3.4
		Total		6.6	6.9	6.7	6.8	6.4	7.1	7.1	7.6	7.3	8.0	8.2	8.1
B512	1	Albumin		1.9	2.3	1.8	1.5	2.3	2.3	2.2	2.2	1.6	2.2	1.9	2.2
		Alpha	Globulin	1.4	1.3	1.3	1.4	1.3	1.3	1.6	1.5	1.6	1.8	1.9	1.5
		Beta	Globulin	0.7	0.6	0.8	0.7	0.7	0.8	0.9	0.9	1.0	1.1	1.1	0.9
		Gamma	Globulin	2.3	2.3	2.5	3.1	2.6	2.7	2.6	2.3	3.0	3.0	3.4	3.2
		Total		6.4	6.4	6.4	6.7	6.9	7.2	7.3	7.1	7.2	8.1	8.2	7.8
B513	1	Albumin		1.9	2.0	1.9	2.2	2.2	2.1	2.3	2.4	2.4	2.2	2.4	2.0
		Alpha	Globulin	1.4	1.4	1.6	1.5	1.5	1.6	1.5	1.4	1.8	1.8	1.6	1.5
		Beta	Globulin	0.8	0.8	0.9	0.9	1.1	0.9	0.9	0.8	0.8	1.0	1.0	1.0
		Gamma	Globulin	2.1	2.1	2.7	2.3	2.1	2.4	2.4	2.4	2.5	3.3	3.0	2.4
		Total		6.2	6.3	7.2	6.9	6.9	7.0	7.2	7.0	7.6	8.2	8.0	6.9
B498	2	Albumin		1.9	1.8	1.6	2.1	1.8	1.7	1.8	1.8	1.3	1.7	1.4	1.8
		Alpha	Globulin	1.2	1.3	1.6	1.2	1.2	1.5	1.3	1.4	1.8	1.6	2.1	1.4
		Beta	Globulin	0.9	0.9	1.0	0.9	1.0	1.0	1.0	1.0	1.2	1.4	1.4	1.6
		Gamma	Globulin	2.2	2.3	2.4	2.0	2.2	2.2	2.2	2.4	2.5	3.0	3.0	2.9
		Total		6.1	6.3	6.6	6.2	6.2	6.5	6.3	6.6	6.7	7.6	7.9	7.7
B500	2	Albumin		1.9	1.7	1.7	1.6	1.6	2.2	2.3	1.9	1.8	1.7	1.7	1.9
		Alpha	Globulin	1.4	1.4	1.2	1.3	1.3	1.6	1.5	1.5	1.5	1.7	1.5	1.5
		Beta	Globulin	0.8	0.9	0.9	0.9	1.0	0.8	0.8	0.8	0.8	1.0	1.0	1.0
		Gamma	Globulin	2.0	2.3	2.4	2.3	1.6	1.9	2.6	2.3	2.4	3.0	2.8	2.8
		Total		6.1	6.4	6.2	6.1	5.4	6.5	6.2	6.5	6.5	7.4	7.0	7.2
B501	2	Albumin		-	1.8	1.6	1.5	1.8	1.8	2.2	1.9	2.1	1.6	1.7	1.8
		Alpha	Globulin	-	1.4	1.3	1.3	1.6	1.5	1.5	1.5	1.6	1.9	2.3	2.0
		Beta	Globulin	-	0.9	0.7	0.8	1.0	0.9	0.9	0.8	0.8	1.1	1.4	1.0
		Gamma	Globulin	-	2.0	2.0	2.0	2.4	2.3	2.2	2.5	2.3	3.0	3.3	3.1
		Total		6.0	6.2	5.5	5.6	6.8	6.6	6.6	6.6	6.8	7.6	8.7	8.0
B504	2	Albumin		2.1	1.7	1.7	2.2	2.5	2.0	2.1	-	1.9	2.1	2.0	2.1
		Alpha	Globulin	1.3	1.5	1.3	1.4	1.6	1.5	1.6	-	2.1	1.6	2.0	1.8
		Beta	Globulin	0.9	0.9	0.8	0.9	1.1	0.9	0.9	-	1.0	1.4	1.2	1.0
		Gamma	Globulin	1.7	2.1	2.3	2.0	2.2	3.0	3.1	-	2.4	3.2	2.9	3.7
		Total		5.9	6.3	6.1	6.6	7.4	7.4	7.7	7.9	7.4	8.3	8.1	8.5
B507	C	Albumin		1.7	1.8	1.7	1.9	-	2.3	2.1	2.1	1.6	1.6	1.6	2.1
		Alpha	Globulin	1.3	1.5	1.5	1.5	-	1.0	1.6	1.6	1.4	1.5	1.8	1.3
		Beta	Globulin	0.8	0.9	0.9	1.0	-	0.7	0.9	0.8	1.0	1.0	1.0	1.2
		Gamma	Globulin	2.1	2.7	2.3	2.3	-	2.1	2.7	2.2	2.2	3.0	2.7	2.5
		Total		6.0	6.9	6.4	6.8	6.2	6.0	7.2	6.6	6.2	7.2	7.1	7.1
B511	C	Albumin		1.9	1.5	2.2	1.7	-	1.7	1.7	1.6	2.0	1.8	1.6	2.0
		Alpha	Globulin	1.3	1.3	1.4	1.3	-	1.5	1.2	1.4	1.3	1.3	1.5	1.2
		Beta	Globulin	0.8	0.8	0.7	0.8	-	1.0	0.9	0.8	1.0	0.9	0.9	0.8
		Gamma	Globulin	2.2	2.4	2.2	2.3	-	2.2	2.7	2.4	2.6	2.4	2.7	2.7
		Total		6.2	5.9	6.6	6.2	6.4	6.4	6.5	6.2	6.8	6.4	6.8	6.6

Appendix Table 13D

Haematological Results, Leucocyte Series

Calf No.		Preinfection		Weeks Post-infection															
				1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18
B498	Neutrophils (band)	60	0	149	121	161	0	64	0	88	0	0	64	0	0	0	0	160	155	0
	Neutrophils (segmented)	1617	2289	3496	1024	2251	1776	1018	2530	2197	1243	1258	1408	2883	1998	2162	1465	2804	1776	1730
	Eosinophils	419	208	372	301	723	1110	826	1061	703	248	398	320	2534	2693	2163	2050	2644	2704	2988
	Basophils	120	0	74	60	0	148	0	82	352	0	0	128	0	174	464	147	160	0	157
	Lymphocytes	9400	10337	10040	9941	12699	11470	10435	11591	13532	10126	10799	10112	11359	12162	9888	9962	9535	10197	10221
	Monocytes	359	1041	744	603	241	296	382	1061	703	608	795	768	699	348	773	1026	732	618	629
	Total leucocytes	11975	13875	14875	12050	16075	14800	12725	16325	17575	12425	13250	12800	17475	17375	15450	14650	16025	15450	15725
B500	Neutrophils (band)	88	79	78	78	80	79	161	275	0	0	0	83	93	84	89	182	88	84	72
	Neutrophils (segmented)	5922	3244	4056	3287	3381	3016	3858	3225	3040	3451	2348	2331	2511	2487	2750	2912	2368	2275	2388
	Eosinophils	177	79	234	704	644	398	643	413	461	1055	606	1166	2232	2573	2219	2366	2457	1938	1520
	Basophils	88	79	156	0	161	79	80	67	92	0	76	83	0	86	89	0	176	84	145
	Lymphocytes	10870	11869	10764	11033	11270	11271	10610	9196	14095	14285	11741	12654	13392	11405	12248	11921	11320	12048	9843
	Monocytes	530	475	312	548	564	1032	723	549	737	384	379	333	372	515	355	819	1141	421	507
	Total leucocytes	17675	15825	15600	15650	16100	15875	16075	13725	18425	19175	15150	16650	18600	17150	17750	18200	17550	16850	14475
B501	Neutrophils (band)	427	50	63	0	225	51	138	293	80	0	82	152	0	0	156	168	101	0	0
	Neutrophils (segmented)	3757	1360	2129	1536	1740	1218	899	1978	2782	788	1875	1138	1359	1676	780	757	1808	1074	1258
	Eosinophils	85	302	564	734	505	760	1659	586	1590	930	1793	1062	3716	3819	3276	4375	6231	4117	4362
	Basophils	0	0	0	0	56	102	69	73	0	72	80	0	0	186	156	252	201	0	0
	Lymphocytes	12038	7758	9456	10346	8194	7714	10230	10841	10971	11317	11573	12368	12144	11920	10218	10852	10553	11456	10484
	Monocytes	768	605	313	734	505	305	830	879	477	1218	897	455	906	1024	1014	421	1106	1253	671
	Total leucocytes	17075	10075	12525	13350	11225	10150	13825	14650	15900	14325	16300	15175	18125	18625	15600	16825	20100	17900	16775
B504	Neutrophils (band)	81	0	0	0	0	0	0	81	0	0	72	0	0	0	74	81	0	0	88
	Neutrophils (segmented)	3639	1959	2450	1961	2604	3066	1273	1788	5387	1787	2312	2262	1817	1918	1770	1953	2258	2113	2275
	Eosinophils	566	346	377	1670	2360	1989	1590	1706	941	298	578	1638	2236	3699	2360	3093	2258	2844	3675
	Basophils	81	0	0	73	81	0	191	81	84	0	72	234	140	69	74	81	80	80	350
	Lymphocytes	11323	8874	6362	10458	10823	10525	9226	12025	10175	7642	10838	10998	9293	7740	10251	10253	11126	10725	10412
	Monocytes	485	346	236	363	407	995	445	569	513	198	578	468	489	274	221	814	403	488	700
	Total leucocytes	16175	11525	9425	14525	16275	16575	12725	16250	17100	9925	14450	15600	13975	13700	14750	16275	16125	16250	17500
B506	Neutrophils (band)	0	60	0	51	68	0	74	68	0	72	32	0	82	0	136	74	0	71	0
	Neutrophils (segmented)	3321	1554	4128	1465	2376	3275	3166	3003	1988	2444	1392	3218	2784	2146	2444	3049	2370	2284	3287
	Eosinophils	615	179	710	1160	950	2128	1030	1228	1224	790	583	1839	2374	1641	2444	3199	1684	2570	2660
	Basophils	0	60	128	202	68	164	147	137	77	72	0	230	82	63	270	149	0	0	78
	Lymphocytes	7995	9440	7740	7070	9706	10480	9277	8873	11169	10422	4403	9578	10316	8459	8077	7809	8046	8779	9312
	Monocytes	369	657	194	152	407	328	1031	341	842	575	65	460	737	316	204	595	375	571	313
	Total leucocytes	12300	11950	12900	10100	13575	16375	14725	13650	15300	14375	6475	15325	16375	12625	13575	14875	12475	14275	15650
B507	Neutrophils (band)	53	0	0	0	55	62	0	0	0	58	68	54	0	54	0	51	0	0	0
	Neutrophils (segmented)	2226	1139	3466	735	1430	1802	1263	1955	1988	1456	1713	1570	862	1411	669	908	1254	1305	1746
	Eosinophils	159	54	60	147	495	808	684	770	435	117	274	217	216	434	382	100	491	681	348
	Basophils	0	0	120	49	0	62	0	59	186	0	0	0	0	54	0	51	0	57	0
	Lymphocytes	7791	8734	8066	8134	8525	9132	8367	8710	8946	9553	11097	8714	9267	8517	8356	8636	8775	8796	7681
	Monocytes	371	923	238	735	495	559	211	356	870	466	548	270	430	380	143	354	380	511	200
	Total leucocytes	10600	10850	11950	9800	11000	12425	10525	11850	12425	11650	13700	10825	10775	10850	9550	10100	10900	11350	9975
B509	Neutrophils (band)	37	172	51	0	52	55	0	51	0	77	37	0	0	0	0	0	0	0	60
	Neutrophils (segmented)	2018	1935	2239	1414	1720	1635	2525	1377	2370	1309	1286	1164	1268	1653	1917	1468	2068	2604	1845
	Eosinophils	150	129	305	576	626	818	968	816	368	385	220	728	1325	1997	3124	3448	2584	2286	1369
	Basophils	38	0	102	105	0	0	0	204	41	0	0	48	115	69	142	147	65	64	60
	Lymphocytes	4896	6149	7173	7961	7558	7685	7203	7293	5069	5544	5586	7372	8414	9780	8662	9318	8014	7620	8090
	Monocytes	336	215	305	419	469	707	54	459	327	385	221	388	403	276	355	294	194	128	476
	Total leucocytes	7475	8600	10175	10475	10425	10900	10750	10200	8175	7700	7350	9700	11525	13775	14200	14675	12925	12700	11900
B510	Neutrophils (band)	108	0	0	77	0	170	0	0	0	0	0	0	0	0	0	0	0	0	89
	Neutrophils (segmented)	1505	1297	2855	2229	2588	2894	5208	1348	1748	1950	2875	2450	1773	2122	1316	2555	1826	2434	2127
	Eosinophils	161	112	225	77	162	170	70	75	83	115	0	189	532	934	485	1058	1111	648	1240
	Basophils	0	113	150	154	0	0	140	75	167	0	0	94	177	85	68	0	79	81	177
	Lymphocytes	8331	9020	10368	11762	12617	13109	8375	12504	13736	19737	17360	15174	14535	13240	11219	13748	12383	12088	13294
	Monocytes	645	733	1427	1076	808	682	282	973	916	1148	1065	943	708	594	762	264	476	974	798
	Total leucocytes	10750	11275	15025	15375	16175	17025	14075	14975	16650	22950	21300	18850	17725	16975	13850	17625	15875	16225	17725
B511	Neutrophils (band)	0	0	66	0	0	0	0	0	0	0	0	0	63	0	52	55	0	0	0
	Neutrophils (segmented)	1785	582	1792	1231	1736	2030	1811	1812	1717	1443	587	1314	1638	2124	1309	2070	1389	1148	1017
	Eosinophils	223	194	266	762	504	572	725	625	120	199	48	400	126	193	210	164	289	287	214
	Basophils	45	48	0	59	56	64	121	0	121	50	98	0	63	65	53	55	58	0	54
	Lymphocytes	6560	8342	10753	9321	8176	9335	9056	9438	9580	7810	8504	9197	10080	9849	8537	8120	9260	9410	9040
	Monocytes	312	534	398	352	728	699	362	625	482	448	538	514	630	644	314	436	579	630	375
	Total leucocytes	8925	9700	13275	11725	11200	12700	12075	12500	12050	9950	9775	11425	12600	12875	10475	10900	11575	11475	10700
B512	Neutrophils (band)	0	36	0	0	99	0	0	0	0	0	0	0	0	0	58	0	0	0	64
	Neutrophils (segmented)	940	1192	1901	595	940	1058	1058	1182	1713	1158	927	997	506	1180	850	678	498	672	2418
	Eosinophils	128	72	371	412	446	370	635	394	497	882	273	586	450	1593	1078	1568	1218	1812	1209
	Basophils	0	0	93	45	50	106	0	49	55	55	55	0	0	0	0	0	111	67	190
	Lymphocytes	7054	5672	6678	7640	7870	8777	8037	7831	8398	8379	9100	9790	9844	8378	9137	7890	8915	10203	8144
	Monocytes	428	253	232	458	495	264	845	394	387	551	545	352	450	649	227	314	333	671	700
	Total leucocytes	8550	7225	9275	9150	9900	10575	10575	9850	11050	11025	10900	11725	11250	11800	11350	10450	11075	13425	12725
B513	Neutrophils (band)	0	0	47	115	0	53	125	0	0	0	45	0	126	0	0	59	0	56	52
	Neutrophils (segmented)	2271	2388	1632	1779	1815	2450	2443	1164	1952	1908	1432	790	1199	1736	2399	998	2153	1973	2038
	Eosinophils	99	47	186	516	110	213	125	48	183	382	402	93	1136	1018	800	823	1722	1184	888
	Basophils	0	48	47	57	0	0	125	0	0	0	45	0	0	0	0	59	0	56	0
	Lymphocytes	6914	6637	7087	8492	8580	7668	9081	7663	9821	9862	6444	8184	9911	8622	7883	9341	7810	7554	6897
	Monocytes	691	430	326	516	495	266	626	825	244	573	582	233	253	599	343	470	615	452	575
	Total leucocytes	9875	9550	9325	11475	11000	10650	12525	9700	12200	12725	8950	9300	12625	11975	11425	11750	12300	11275	10450

All results expressed in numbers of cells per cubic millimetre of blood

Appendix Table 13E Haematological results, erythrocyte series

Packed Cell Volume (%)

Group	Calf No.	Preinfection		Weeks post-infection 8	12	18
2	B498	32	32	36	35	39½
	B500	35	35½	37	36½	31
	B501	36½	36½	38½	37½	46½
	B504	34	33	36½	31½	38½
1	B506	33	37	36½	34	35
	B509	31½	31	29½	32½	32½
	B512	35½	38½	39	35	38
	B513	32½	31½	35	35½	31½
C	B507	35	33½	36½	36½	33½
	B511	33	32	33½	33½	31½

Haemoglobin (G./100 ml.)

Group	Calf No.	Preinfection		Weeks post-infection 8	12	18
2	B498	10.1	10.3	11.7	11.2	12.7
	B500	11.0	11.3	11.9	12.1	9.7
	B501	11.3	11.4	11.9	11.9	14.3
	B504	11.3	11.1	12.1	10.9	12.6
1	B506	9.8	11.4	11.5	10.8	10.9
	B509	10.3	10.3	10.0	10.5	10.9
	B512	10.9	12.1	12.5	11.3	11.7
	B513	9.8	10.2	10.9	11.0	10.0
C	B507	11.0	10.9	11.8	11.9	10.7
	B511	10.2	10.2	10.8	10.6	9.9

Red Cells (million/cmm.)

Group	Calf No.	Preinfection		Weeks post-infection 8	12	18
2	B498	8.14	7.94	-	-	9.78
	B500	8.28	7.04	-	-	6.71
	B501	8.63	9.47	-	-	9.81
	B504	8.22	7.98	-	-	8.18
1	B506	7.63	6.90	-	-	7.08
	B509	7.66	7.02	-	-	6.73
	B512	7.34	8.93	-	-	8.94
	B513	6.79	6.37	-	-	6.27
C	B507	8.68	7.70	-	-	7.57
	B511	8.70	8.09	-	-	7.89

Mean Corpuscular Haemoglobin Concentration (%)

Group	Calf No.	Preinfection		Weeks post-infection 8	12	18
2	B498	31.4	32.2	32.4	32.1	32.1
	B500	31.5	31.7	32.3	33.0	31.4
	B501	31.0	31.3	31.0	31.7	30.7
	B504	33.3	33.8	33.0	34.7	32.7
1	B506	29.7	30.9	31.5	31.6	31.0
	B509	32.8	33.4	33.8	32.4	33.4
	B512	30.7	31.5	32.1	32.4	30.8
	B513	30.1	32.4	31.3	31.1	31.8
C	B507	31.5	32.5	32.3	32.6	32.0
	B511	37.9	39.5	32.2	31.7	31.3

Mean Corpuscular Volume (c.μ.)

Group	Calf No.	Preinfection		Weeks post-infection 18
2	B498	39.3	40.3	40.4
	B500	42.3	50.4	46.2
	B501	42.3	38.6	47.4
	B504	41.4	41.4	47.1
1	B506	43.3	53.6	49.4
	B509	41.1	44.2	48.3
	B512	48.3	43.1	42.5
	B513	47.8	49.5	50.2
C	B507	40.3	43.5	44.3
	B511	37.9	39.5	39.9

Mean Corpuscular Haemoglobin (μ.μ.g.)

Group	Calf No.	Preinfection		Weeks post-infection 18
2	B498	12.4	13.0	13.0
	B500	13.3	16.0	14.5
	B501	13.1	12.1	14.6
	B504	13.8	14.0	15.4
1	B506	12.8	16.6	15.3
	B509	13.5	14.7	16.1
	B512	14.8	13.6	13.1
	B513	14.4	16.1	16.0
C	B507	12.7	14.2	14.2
	B511	11.7	12.6	12.5

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STUDIES ON THE AETIOLOGY OF ANAEMIA IN CHRONIC FASCIOLIASIS IN SHEEP

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SUMMARY

The erythrocytes of four sheep having chronic infections with *Fasciola hepatica* and two uninfected controls were labelled with ^{51}Cr. The resulting radioactivity in the faeces was used to give an estimate of the daily loss of blood. The apparent loss of blood in the faeces of the infected sheep correlated closely with the fluke burden, the calculated daily faecal blood loss per fluke being 0·5–1·0 ml. The total daily mean losses in the infected sheep ranged from 42 ml. to 189 ml. compared with only 2 ml. to 4 ml. in the uninfected controls.

Red-cell-survival curves were obtained for the same sheep using DF ^{32}P as a red cell label. The physiological destruction of senile erythrocytes in the control animals gave a linear relationship between time, and the residue of the initially labelled red cells. In the infected animals however, the loss was curvilinear and it could be shown that this deviation from the normal would have resulted from a daily loss of blood of the same order as that previously demonstrated in the faeces. In the most heavily infected animals, the blood loss estimated from the survival curves was rather greater than that found in the faeces. This may have resulted from a reduced life for the poikilocytes and macrocytes in the blood of these animals.

Measurements of the activity of the bile from the gall bladders of these sheep suggested that the faecal blood was originating from the liver.

The amount and activity of the erythropoietic bone marrow was increased in the infected sheep as compared with the controls. This caused a considerable increase in the number of red cells produced each day.

It is concluded that the anaemia of chronic fascioliasis is primarily caused by loss of blood into the bile ducts.

INTRODUCTION

Anaemia may result from one or more of three processes. These are haemorrhage—an acute or chronic loss of whole blood or intact red cells; haemolysis—a pathological, intravascular destruction of red cells as opposed to the physiological destruction of senile red cells; and dyshaemopoiesis—a pathological reduction in the rate of production of new red cells.

Adult *Fasciola hepatica* have usually been assumed to feed by ingesting blood, so causing a blood loss type of anaemia. However, some recent studies have

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suggested that they browse on the hyperplastic biliary epithelium (Dawes, 1963), and that the anaemia results from a relative dyshaemopoiesis and from a reduction in red cell life (Sinclair, 1964, 1965). The present studies were designed to give direct evidence of the amount of any blood loss in the faeces resulting from infection with *F. hepatica*, and also to demonstrate whether such blood loss was occurring into the bile ducts, and could account for the severity and nature of the anaemia observed.

MATERIALS AND METHODS

Experimental animals

Six 10-month-old Cheviot wethers from a farm with no history of fascioliasis, were brought indoors and dosed on two occasions with 100 mg. of thiabendazole per kg. live-weight. They were thereafter kept on a litter of wood shavings, and the pens were cleaned twice weekly so as to minimize re-infection with parasitic nematodes. The sheep were fed on a daily ration of hay and water *ad lib.*, plus 0·75 lb. increasing to 1·5 lb. per head of a 4:1 mixture of barley and Cattle and Sheep Protein Concentrate Pellets (Scottish Agricultural Industries). Repeated faecal examinations before and during the experiment showed no evidence of trematode infections—except for the experimental infections—and only very small numbers of coccidial oocysts and occasional *Strongyloides* and *Trichuris* eggs in some of the sheep. The virtual absence of nematode infections was confirmed when the contents of the abomasum and the intestines were examined in detail after death.

Experimental design

At 11–12 months of age two of the sheep (Nos. 20 and 23) were selected at random and each infected with 2400 metacercariae of *F. hepatica*, two (Nos. 24 and 25) were similarly selected and infected with 600 metacercariae each, while the remaining two (Nos. 13 and 14) were left as uninfected controls. The metacercariae were produced in laboratory cultures of *Lymnaea truncatula* infected with miracidia from *Fasciola hepatica* eggs obtained from the gall bladders of naturally infected sheep. The techniques used were derived from those described by Kendall & McCullough (1951) and by Kendall (1953).

The studies using radiochemical tracers commenced 5 months after infection and the sheep were killed 7 months after infection. At post-mortem examination the livers were dissected, and the flukes in the bile ducts were counted. A few flukes were also found in the small intestine in three of the sheep. However it is probable that this resulted from the handling of the liver, as no flukes were found in the small intestine when care was taken to ligate the common bile duct before further handling.

Haematological methods

Packed cell volumes were determined at weekly intervals using a Hawksley microhaematocrit centrifuge and reader. Other haematological examinations were carried out at 3-weekly intervals. Haemoglobin concentrations were

estimated by an alkaline haematin method (Clegg & King, 1942), and the red cell count by the use of an electronic cell counter (Coulter Electronics Ltd, Model A, Medical).

At post-mortem examination, the sternum, one femur, one ileum, and four to six lumbar vertebrae were bisected, and were then photographed, so as to display the extent of the erythropoietic tissue in the marrow cavities.

Colour transparencies of the bones were used to draw enlarged diagrams of the femurs onto card, and to delineate the area of the red marrow inside the femurs. An estimate of the difference in the amount of active marrow in these bones could then be obtained by cutting out and weighing firstly the whole bone, and secondly the smaller area of the active marrow, so that the ratio of the two areas could be calculated.

Methods using ^{51}chromium

Sodium chromate (^{51}Cr) solution BP was obtained from the Radio Chemical Centre, Amersham. Each sheep received an accurately known volume of autologous, washed red cells, which had been labelled with 120 μc of the ^{51}Cr by the method described by Dacie & Lewis (1963). This injection was given through an intravenous catheter so as to minimize any risk of the labelled cells leaking from the vein at the injection site. The total blood volume was determined from a blood sample taken 10 minutes after injection (Dacie & Lewis, 1963).

Twenty-four hour faecal samples were collected from the sheep using bags and harness. The individual samples were weighed, and the fresh faeces were thoroughly mixed with a spatula and packed into a polythene counting jar to a constant volume of 350 ml. The weight of faeces contained in this volume varied from 310 to 380 g., but the geometry of the system remained constant. For comparison with these faecal samples, 10 ml. of whole blood from the same animal was diluted in distilled water in a similar jar to the same volume.

A double scintillation system (Dacie & Lewis, 1963) was used for counting these bulky samples. A 2¼ inch diameter thallium-activated sodium iodide well-type crystal was sited beneath the sample and a similar 1½ inch diameter end-on crystal in close approximation above the sample. Both the crystals and the sample were screened with lead shielding. The signals from the two photomultipliers associated with the crystals were led into a single pre-amplifier and scaler (Panax Equipment Ltd). Counts on whole blood samples and on decay standards were carried out using 7 ml. aliquots in the well-type crystal alone, the lead to the end-on counter being disconnected.

The time taken for the ingesta to pass from the duodenum into the faeces of sheep has been estimated at about 24 hours (Benzie & Phillipson, 1957), so each faecal count was related to the previous day's blood count, after this had been corrected for 24 hours' decay. The same apparatus and settings were used for both counts. The count from the faeces was also corrected to allow for the activity present in normal sheep faeces (Brambell, Charleston & Tothill, 1964).

The total blood lost in the faeces in 24 hours could then be derived from the following formula:

Faecal blood content (ml.) =

$$10 \times \frac{(\text{corrected faecal count}) \times (\text{total weight of faeces})}{(\text{corrected previous days blood count}) \times (\text{weight of faeces counted})}.$$

The mean daily loss of blood in the faeces was calculated in this way from determinations made on five consecutive days commencing 2 days after the labelled cells had been given.

Methods using DF ^{32}P

Di-isopropyl phosphorofluoridate-^{32}P (DF ^{32}P) sterile solution in propylene glycol, was obtained from the Radio Chemical Centre, Amersham, and each sheep received 120 μc in 0·6 ml. by intramuscular injection. Successive 10 ml. blood samples were obtained at 1–3 day intervals over a total period of 32–36 days. The whole blood samples were lysed with saponin, and their activity counted in a liquid GM counter (Twentieth Century Electronics Ltd, N.6 Liquid Counter). To ensure that the DF ^{32}P had been completely incorporated into the red cells, the 100 per cent origin for the survival curves was taken as 2 days after the injection was given. Even so, the curves suggested that labelling was still occurring after this time.

DF ^{32}P does not elute from sheep red cells, and is not re-utilized when they are destroyed. Hence the regression between the residue of labelled red cells and time is linear, in the absence of random loss of red cells by blood loss or haemolysis (Eadie, Smith & Brown, 1960). The regression therefore takes the form: $R = C - at$, where R = the residue of the original labelled cells, C = the total initial number of red cells in the sheep, t = the elapsed time in days since labelling, and a = the mean daily physiological loss of red cells due to senility.

However, in the presence of random blood loss—even if this only amounts to 1 per cent of the total blood volume daily—the loss due to senescence is greatly reduced. The regression is then approximately exponential, so that $R = Cb^t$ and $c = C - Cb$, where c = the mean daily random loss of red cells, and b is a constant.

RESULTS

Haematology

The packed cell volumes for the sheep throughout the experiment are shown in Fig. 1. Similar results to these were obtained for the haemoglobin concentrations and for the red cell counts. There were no significant changes in the mean corpuscular volume, the mean corpuscular haemoglobin, or the mean corpuscular haemoglobin concentration, in the infected or in the control sheep throughout the course of the experiment. There was severe poikilocytosis, anisocytosis, and punctate basophilia in the red cells from the heavily infected sheep (Fig. 2).

The area of erythropoietic activity was markedly increased in the marrow cavities of the infected sheep, as compared with the controls (Figs. 3 and 4). This difference was most noticeable in the femurs which showed little erythro-

poietic activity in the normal animals, but considerable activity in the infected animals (Table I).

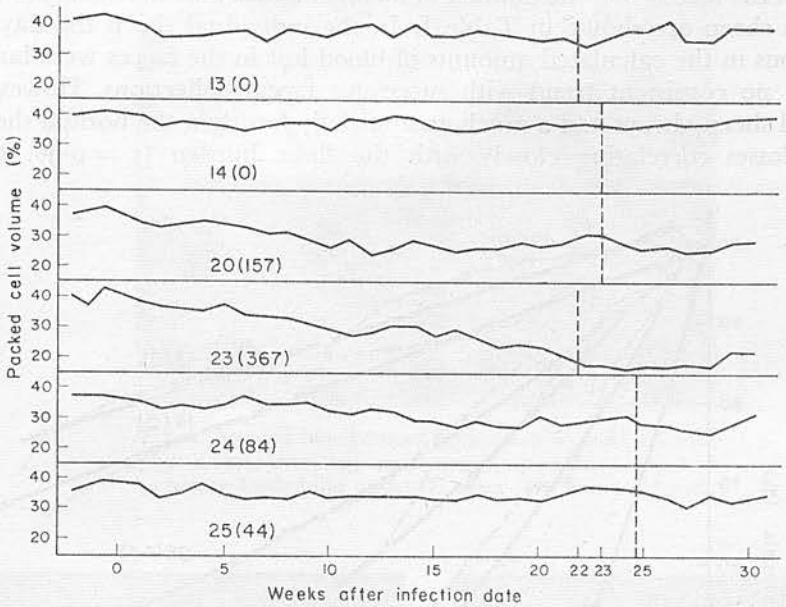

Fig. 1. Variations in the packed cell volumes in control and infected sheep during the course of the experiment. The vertical broken lines indicate the time at which the radio-tracer studies commenced in each sheep. The figures in parentheses show the fluke burden in each sheep.

TABLE I

RESULTS OF STUDIES ON THE AETIOLOGY OF THE ANAEMIA IN CHRONIC FASCIOLIASIS IN SHEEP

	Sheep No. 23	20	24	25	13	14
No. of flukes found *post mortem*	367	157	84	44	0	0
Total blood volume (l.)	2·95	2·68	2·09	3·41	2·62	2·94
Counts/min./ml. of bile *post mortem*	2·3	2·4	6·9	2·4	0·8	0·4
Estimated daily random blood loss (ml.) — From faecal ^{51}Cr studies	189	86	69	42	2	4
Estimated daily random blood loss (ml.) — From red-cell survival studies	324	142	66	74	—	—
Estimated daily random blood loss (ml.) — From activity of bile	396	55	86	36	3	4
Estimated daily production of red cells from ^{51}Cr studies ($\times 10^{10}$)	104	63	61	45	25	32
Percent cross sectional area of erythropoietic marrow to cross sectional area of femur	29	15	14	12	3	6

Studies using ^{51} *chromium*

The estimated total blood volumes of the sheep, the apparent daily loss of blood in the faeces, and the number of flukes found at post-mortem examination in each sheep are shown in Table I. In the individual sheep the day-to-day variations in the calculated amounts of blood lost in the faeces were large, but showed no consistent trend with successive faecal collections. However, the infected sheep always had a much greater daily loss than the normal sheep, the mean losses correlating closely with the fluke burden ($r = 0{\cdot}96$, $t = 6{\cdot}5$,

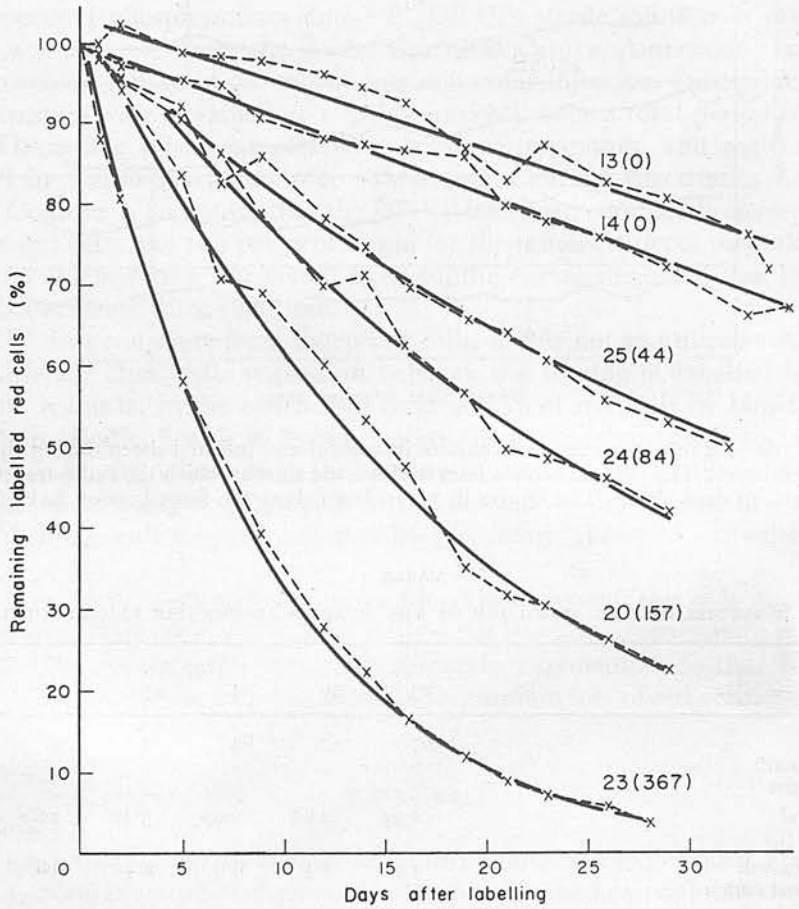

Fig. 5. Red-cell-survival curves of control sheep, and sheep with chronic fascioliasis. The figures given on the curves are the numbers of the respective sheep, followed by the fluke burden in parentheses. The thin broken lines join the experimental plots, and the thicker solid lines show the theoretical curves derived from these figures using the following formulae:

Sheep 13	$S = 103{\cdot}4 - 0{\cdot}8162t$
Sheep 14	$S = 100{\cdot}3 - 0{\cdot}9551t$
Sheep 20	$S = 106{\cdot}6\ (0{\cdot}9471^t)$
Sheep 23	$S = 104{\cdot}8\ (0{\cdot}8904^t)$
Sheep 24	$S = 103{\cdot}7\ (0{\cdot}9685^t)$
Sheep 25	$S = 100{\cdot}7\ (0{\cdot}9781^t)$

PLATE I

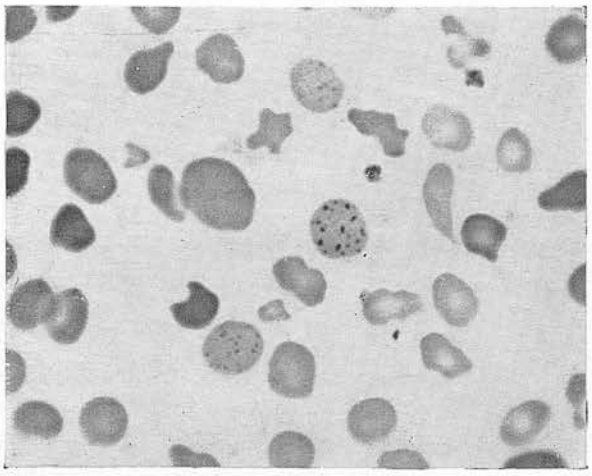

Fig. 2. Stained blood smear from sheep 23 infected with 367 flukes, showing anisocytosis, poikilocytosis, and punctate basophilia 30 weeks after infection (× 1200).

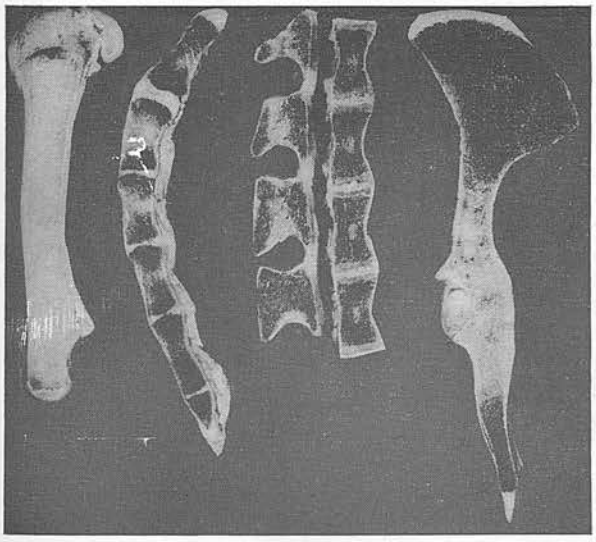

Fig. 3. Displayed marrow cavities of the sternum, lumbar vertebrae, one femur and one ileum from the uninfected sheep 13. All these sites show white areas where adipose tissue has replaced the erythropoietic marrow.

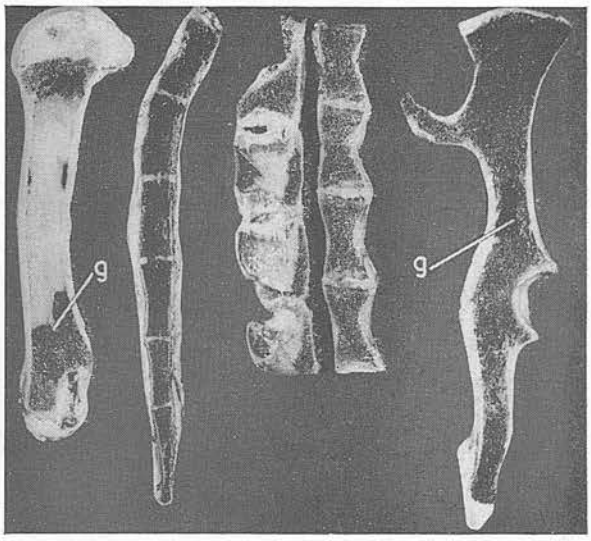

Fig. 4. Displayed marrow cavities of the sternum, lumbar vertebrae, one femur, and one ileum from sheep 23, infected with 367 flukes. The only area showing adipose tissue is in the femur and this is greatly reduced compared with the uninfected animal. The black areas in the femur and ileum (g) are areas of very cellular gelatinous erythropoietic tissue.

$P < 0{\cdot}01$). There was also some indication that there may be an inverse correlation between the number of flukes in the liver and the daily blood loss per fluke ($r = -0{\cdot}83$, $t = 3{\cdot}79$, $P < 0{\cdot}1$).

Studies using DF ^{32}P

The red-cell-survival curves for each of the sheep are shown in Fig. 5.

The daily loss of blood by the uninfected animals had been shown by the ^{51}Cr studies to be very slight, and as expected the survival curves for these animals were linear ($r > -0{\cdot}96$ in each case). The average potential red cell life calculated from these regressions was 123 days for No. 13, and was 105 days for No. 14. Both of these figures are within the range given by previous authors (Eadie *et al.*, 1960; Tucker, 1963).

The curves calculated from the data for each of the infected sheep by the exponential approximation are also shown in Fig. 5, and the mean daily loss of blood from each sheep, calculated in this way is shown in Table I. The calculated curves correlated extremely closely with the experimental figures ($r > -0{\cdot}98$ in each case).

The ^{32}P activity of the bile in the gall bladders of the sheep was measured after they had been killed. In all cases this was low (Table I), but because the activity of the blood in the infected sheep was by now much lower than that in the blood of the uninfected sheep, the loss of blood into the bile necessary to cause these activities was much greater in the infected animals than in the controls. It is not possible to obtain an accurate estimate of the daily loss of blood in the bile from these figures, because the daily production of bile is very variable (Quinn, 1936). However, if the mean daily bile production is estimated at 11 ml./kg. live-weight per day, as in the sheep studied by Quinn, the daily loss of blood in the bile is of the same order as the losses shown by the other methods (Table I).

DISCUSSION

It has been shown in man that ^{51}Cr is mainly excreted in the urine, and is neither excreted into the intestine nor absorbed from the intestine in more than minimal amounts, so that it is suitable for studies on blood loss in the faeces (Roche, Perez-Gimmenez, Layrisse & Diprisco, 1957). Similar studies have not been conducted in sheep, but there is no indication that these considerations do not also apply in these animals.

However, it is known that a proportion of the ^{51}Cr label rapidly elutes from sheep red cells *in vivo*, at a rate which varies from animal to animal (Tucker, 1963). Sinclair (1967) has suggested that this elution occurs more rapidly from the red cells of sheep infected with *F. hepatica*, and that a higher proportion of the eluted chromium is excreted in the bile in infected sheep than in uninfected sheep. Nevertheless the residual activity in the blood of the infected sheep in this experiment did not suggest that there had been an additional increase in the rate of urinary excretion commensurate with that observed in the faeces. It is therefore unlikely that these effects account for much of the ^{51}Cr which occurred in the faeces of these sheep.

Thus despite the theoretical arguments of Dawes (1963), in which he queries the possibility of the flukes in the bile ducts gaining access to blood, it would appear from the results obtained in these studies, that there is a considerable loss of red cells into the faeces during chronic fascioliasis. This supports earlier findings by Holmes *et al.* (1967), and Jennings, Mulligan & Urquhart (1956) using radio-tracers, by Stephenson (1947) who demonstrated the presence of intact red cells within the caecal contents of *F. hepatica*, and by Todd & Ross (1966), who showed by spectroscopic methods that the black caecal contents of *F. hepatica* contained degradation products of haemoglobin. In view of the associated hypoalbuminaemia (Sinclair, 1962), and the demonstrable loss of labelled albumin and other plasma macromolecules in the faeces (Dargie, Holmes, McLean & Mulligan, 1967), it is likely that this loss occurs in the form of whole blood. Furthermore, the present studies indicate that the loss of red cells labelled with DF ^{32}P into the bile in chronic fascioliasis is sufficient to account for the blood content of the faeces.

Unfortunately, because ^{51}Cr rapidly elutes from the red cells, it does not provide a suitable label for obtaining red-cell-survival curves in sheep. However, DF ^{32}P is a suitable label for this use (Eadie *et al.*, 1960). The change in the shape of the survival curves for red cells labelled with DF ^{32}P in sheep with chronic fascioliasis as compared with uninfected sheep (Fig. 5), can be explained in terms of chronic haemorrhage, or in terms of a haemolytic effect. A reduction in the intrinsic average potential red cell life would not cause this exponential type of survival curve, but would result in an increased slope for the normal linear regression (Harris, 1963). The estimates of the daily random blood loss from these curves are of the same order as those obtained by the other methods (Table I), but in the most heavily infected sheep, they tend to be higher than the direct ^{51}Cr estimates of the blood loss in the faeces. It would thus appear that some haemolysis may be occurring, which may be associated with the severe poikilocytosis and anisocytosis seen in heavy infections, as poikilocytes and macrocytes are believed to be more fragile than normal red cells (Prankerd, 1961).

Despite this daily random blood loss the infected sheep maintained their haematological values over a period of several weeks while the radio-tracer studies were in progress (Fig. 1). This suggests that there is a compensatory absolute increase in erythropoiesis which is replacing the lost red cells (Table I), and this is clearly supported by the findings in the bone marrow. Sinclair (1964) suggested that dyshaemopoiesis is a major factor in the aetiology of the anaemia of fascioliasis. He was in part basing his deduction on the greater severity of the anaemia in his infected animals as compared with that shown by sheep from which he had removed daily amounts of blood, which were supposedly similar to the amount lost each day due to the flukes. However, he used a figure for the daily blood loss per fluke of only 0·2 ml., taking this from work described by Urquhart, Mulligan & Jennings (1954). This figure would appear to be too low, and in a later paper Sinclair (1965) gives the results of an experiment in which larger volumes of blood were removed from sheep each day. In this latter case the level of anaemia was very similar to that which would have

been caused by an infection with *F. hepatica* giving the same total daily blood loss, if the loss per fluke was at the higher rate suggested in this present study. Sinclair took no account of changes in the total amount of erythropoietic tissue, but his cytological findings in the marrow, and his studies on the uptake of of radio-iron both suggest that there is an absolute increase in erythropoietic activity, as the changes occurred in the same direction in the bled sheep and in the sheep infected with *F. hepatica*. Symons & Boray (1967), also using ^{59}Fe, concluded that the rate of erythropoiesis was greatly increased in sheep infected with *F. hepatica*, probably to a maximum. It would thus appear that dyshaemopoiesis is not a major aetiological factor in the anaemia of chronic fascioliasis of sheep, although the possibility that there may be a relative dyshaemopoiesis, following the prolonged over-stimulation of the haemopoietic tissues, cannot be ruled out.

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Etudes de l'éthiologie de l'anémie dans la fasciolase des moutons

(Sewell *et al.*)

Résumé. Les érythrocytes de quatre moutons atteints d'infection chronique par le *Fasciola hepatica* et ceux de deux moutons de contrôle non infectés ont été marqués avec du ^{51}Cr. La radioactivité que l'on a retrouvée dans les matières fécales a été utilisée pour évaluer les pertes de sang journalières. Les pertes de sang apparentes dans les matières fécales des animaux infectés correspondaient de près au degré d'infection, la perte de sang journalière dans les fecès étant calculée à 0,5–1 ml. par douve. Le total des pertes journalières moyennes des moutons infectés était compris entre 42 ml. et 189 ml., en comparaison avec 2 ml. à 4 ml. chez les animaux de contrôle non infectés.

Des courbes de survie des globules rouges ont été établies pour les mêmes moutons après marquage au ^{32}P. La destruction physiologique des érythrocytes sénils chez les animaux de contrôle a donné des courbes linéaires pour les rapports entre le temps et les résidus des globules rouges marqués à l'origine. Toutefois, chez les animaux infectés, les pertes prenaient la forme d'une courbe curviligne et on a pu démontrer que cette déviation de la normale était due à des pertes journalières de sang du même ordre que celles qui avaient été enregistrées précédemment par l'examen des matières fécales. Dans les cas d'animaux gravement atteints, les pertes de sang évaluées après l'établissement des courbes de survie apparaissaient quelque peu plus élevées que ne l'indiquait l'examen des fecès. Il est possible que ce fait soit dû à une diminution de la vie des poikilocytes et des macrocytes dans le sang de ces animaux.

Les mesures de l'activité de la bile venant de la vésicule biliaire de ces animaux ont suggéré que le sang contenu dans les matières fécales provenait du foie.

La quantité et l'activité de la moelle osseuse érythropoietique étaient plus élevées chez les animaux infectés que chez les moutons de contrôle. Il en résultait un accroissement considérable du nombre de globules rouges produit chaque jour.

En conclusion, on peut dire que l'anémie de la fasciolase chronique est due principalement à une perte de sang dans les canaux biliaires.

Studien zur Ätiologie der Anämie bei der chronischen Fasciolosis von Schafen

(Sewell *et al.*)

Zusammenfassung. Die Erythrozyten von vier Schafen, die an einer chronischen Infektion mit *Fasciola hepatica* litten, und die von zwei nicht infizierten Kontrollen, wurden mit ^{51}Cr markiert. Aus der sich ergebenden Radioaktivität der Faeces wurde der tägliche Blutverlust errechnet. Der Blutverlust infizierter Schafe durch den Darm, entsprach weitgehend der Schwere der Leberegelinfektion und der tägliche Blutverlust betrug 0,5–1,0 ml. pro Leberegel. Im Durchschnitt betrugen die gesamten Blutverluste der infizierten Schafe von 42 ml. bis 189 ml., verglichen mit nur 2 ml. bis 4 ml. der nicht infizierten Kontrolltiere.

Die Überlebenskurve der roten Blutkörperchen derselben Schafe wurde durch Markierung mit ^{32}P festgestellt. Der physiologische Abbau überalterter Erythrozyten der Kontrolltiere ergab ein lineares Verhältnis zwischen der Zeit und dem Rest der anfangs markierten roten Blutkörperchen. Für infizierte Tiere ergab sich jedoch eine Kurve, und es konnte bewiesen werden, dass diese Abweichung vom Normalen das Ergebnis von Blutverlusten der gleichen Grösse ist, die in den Faeces festgestellt worden waren. Für Tiere mit sehr schweren Infektionen wurden aus der Überlebenskurve der Erythrozyten höhere Blutverluste errechnet als aus den Faeces. Dies könnte auf eine herabgesetzte Lebensdauer der Poikilozyten und Makrozyten im Blut der Tiere zurückzuführen sein.

Messung der Radioaktivität der aus der Gallenblase dieser Schafe gewonnenen Galle deutet darauf hin, dass das in den Faeces festgestellte Blut aus der Leber stammt. Die Menge und Aktivität des erythropoëtischen Knochenmarks infizierter Schafe war gegenüber gesunden Kontrolltieren erhöht. Hierdurch war auch die Zahl der täglich freigesetzten roten Blutkörperchen erheblich erhöht.

Man schliesst daraus, dass die Anämie von chronisch mit Faciolae infizierten Schafen auf Blutverlust durch die Gallengänge zurückzuführen ist.

Estudios de la etiología de la anemia en la fascioliasis crónica del ganado lanar

(Sewell *et al.*)

Resumen. Se etiquetaron con ^{51}Cr los eritrocitos de cuatro ovejas que tenían infecciones crónicas de *Fasciola hepatica*, y asimismo dos controles no infectados. La resultante radio-

actividad de las heces se utilizó para llegar a una evaluación de la pérdida diaria de sangre. Le pérdida aparente de sangre en las heces de las ovejas infectadas estaban en estrecha correliación con la carga de trematodos, habiéndose calculado que la pérdida fecal diaria de sangre por trematodo es de 0,5 a 1,0 ml. Las pérdidas medias totales diarias en las ovejas infectadas oscilaban entre 42 y 189 ml. mientras que en los controles no infectados no eran más que de 2 a 4 ml.

Se consiguieron curvas de sobrevivencia de los corpúsculos rojos de las mismas ovejas usando ^{32}P como etiqueta de las células rojas. La destrucción fisiológica de los eritrocitos seniles en los animales de control proporcionó una relación lineal entre el tiempo y el residuo de las células rojas inicialmente etiquetadas. En cambio, en los animales infectados la pérdida era curvilineal, pudiéndose demostrar que esta desviación con respecto a la normal habría resultado de una pérdida diaria de sangre del mismo orden que la anteriormente demostrada en las heces. En los animales más fuertemente infectados, la pérdida de sangre que se estimó a base de las curvas de sobrevivencia era algo mayor que la que se encontró en las heces. Esto puede haber sido resultado de una reducción en la vida de los poiquilocitos y macrocitos en la sangre de estos animales.

La medición de la actividad de la bilis procedente de la vesícula biliar de estas ovejas sugirió que la sangre de las heces tenía su origen en el hígado.

La cantidad y la actividad de la médula de hueso eritopoyética aumentó en las ovejas infectadas en comparación con los controles. De ello resultó un aumento considerable en el número de células rojas producidas cada día.

Se concluye que la anemia de la fascioliasis crónica se produce primordialmente por pérdida de sangre que va a parar a los conductos biliares.

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